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The regulation of oestradiol and progesterone concentrations
in the female reproductive tissues

by Satish Batra

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THE REGULATION OF OESTRADIOL AND PROGESTERONE CONCENTRATIONS IN THE FEMALE REPRODUCTIVE TISSUES

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PURPOSE: 1. To develop methods for the determination of plasma concentrations of free and protein-bound progesterone and for the determination of estradiol 17- β (E2) and progesterone (P) concentrations in small samples of reproductive tissue from humans, rabbits and guinea-pigs. 2. To elucidate mechanisms controlling steroid hormone concentrations in the target tissues, and the interactions of E2 and P at the cellular and receptor level.

METHODS: Free P was determined by its adsorption to membrane filters. Plasma and tissue E2 and P were determined by radioimmunoassays. However, tissues were first digested with a mixture of a detergent and NaOH before the extraction of steroids with an organic solvent.

RESULTS: There was in general a lack of relationship between tissue and plasma concentrations. Whereas the concentration of E2 in the human myometrium was significantly influenced by the distance from the placenta, the P concentration was not. Uterine E2 decreased significantly during pseudopregnancy in the rabbit in spite of relatively constant level in the plasma. Although tissue and cytosolic P concentrations in the rabbit myometrium increased after P treatment, P receptors levels decreased markedly. Both tissue E2 and E2 receptors decreased after P treatment. The significance of the findings in the control of tissue hormonal concentrations and their possible implications in the initiation of parturition is discussed

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**The regulation of oestradiol and progesterone
concentrations in the female
reproductive tissues**

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University of Lund, Lund, Sweden



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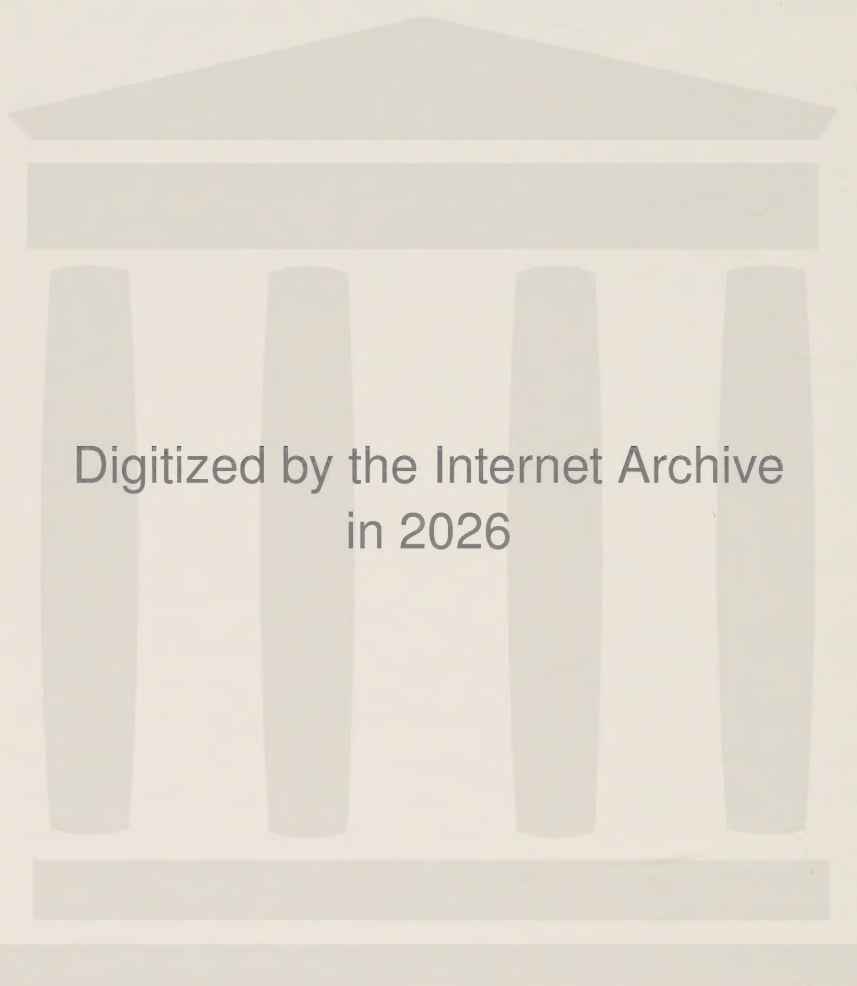
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ORIGINAL PAPERS

This thesis is based on the following publications, which will be referred to in the text by their Roman numerals:

- I. Batra, S.: A new and improved method for the separation of free from protein-bound progesterone. *J Endocrinol* 62:537, 1974.
- II. Batra, S.: Aqueous solubility of steroid hormones: An explanation for the discrepancy in the published data. *J Pharm Pharmacol* 27:777, 1975.
- III. Batra, S.: New simplified procedures for the determination of progesterone by competitive protein binding and radioimmunoassay. *J Steroid Biochem* 7:131, 1976.
- IV. Batra, S., Bengtsson, L.Ph., Grundsell, H. & Sjöberg, N.-O.: Levels of free and protein-bound progesterone in plasma during late pregnancy. *J Clin Endocrinol Metab* 42:993, 1976.
- V. Batra, S. & Grundsell, H.: Studies on certain aspects of progesterone and cortisol dynamics before, during and after labor. *Clin Endocrinol* 8:403, 1978.
- VI. Batra, S. & Bengtsson, L.Ph.: A highly efficient method for the extraction of progesterone from uterus and its compatibility with subsequent radioimmunoassay. *J Steroid Biochem* 7:599, 1976.
- VII. Batra, S.: Unconjugated estradiol in the myometrium of pregnancy. *Endocrinology* 99:1178, 1976.
- VIII. Batra, S. & Bengtsson, L.Ph.: 17β -estradiol and progesterone concentrations in the myometrium of pregnancy and their relationships to the concentrations in the peripheral plasma. *J Clin Endocrinol Metab* 46:622, 1978.
- IX. Batra, S., Bengtsson, L.Ph. & Sjöberg, N.-O.: Interrelations between plasma and tissue concentrations of 17β -oestradiol and progesterone during human pregnancy. Submitted for publication.
- X. Batra, S., Helm, G., Öwman, Ch., Sjöberg, N.-O. & Waller, B.: Female sex steroid concentrations in the ampullary and isthmic regions of the human Fallopian tube and their relationship to the plasma concentrations during the menstrual cycle. Submitted for publication.
- XI. Batra, S., Öwman, Ch., Sjöberg, N.-O. & Thorbert, G.: Relationship between plasma and uterine oestradiol in pseudopregnant rabbits. *J Reprod Fertil*. In press.
- XII. Batra, S. & Källstrand, K.: Are there cyclic variations in estradiol secretion in the non-pregnant rabbit? *Experientia*. In press.

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- Batra, S., Sjöberg, N.-O. & Thorbert, G.: Sex steroids in plasma and reproductive organs of the female guinea-pig. Submitted for publication.



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INTRODUCTION

The complex mechanisms whereby hormones regulate, organize and manipulate the entire organism are only just beginning to be appreciated. Since the characterization and identification of the steroid hormones in the first half of this century, recent research effort has placed considerable emphasis on discovering both how steroid biosynthesis in endocrine tissues is controlled, and the details of the intracellular mechanisms by which the steroid hormones act within their target tissues. The elucidation of the mode of action of steroid hormones at the cellular and molecular levels is one of the most challenging areas of present day research.

The many and diverse physiological effects of oestrogen and progesterone are well known. Initial work concentrated on studying the effects of steroid hormones administered in vivo in terms of changes in enzyme activities, uptake of small molecules and synthesis of macromolecules such as nucleic acids, proteins and phospholipids in cells of target organs. These studies have helped to elucidate several biochemical events, such as the DNA dependent sequential synthesis of RNA and proteins brought about by estrogens (36,44,45). However, a different approach was required in order to examine the molecular interaction of the hormone itself with its target cell.

With the introduction of tritiated steroid hormones of high specific activity, it became possible not only to localize hormones at the cellular level but also to determine very low concentrations of steroids, such as those occurring in the peripheral plasma.

Thus in the sixties great strides were made in the development of radioimmunoassays, competitive protein binding assays, and radioligand receptor assays, collectively called saturation analysis techniques (15). As a result a vast amount of quantitative data has become available on the concentrations of oestrogens and progesterone in the plasma of various animal species, including man, under physiological, pathological and experimental conditions. Furthermore, a large number of studies have dealt with the binding of oestrogen and progesterone to proteins in target tissues such as the uterus. Relatively little is known, however, about the concentration of oestradiol and progesterone in the myometrium and other reproductive tissues. This situation by no means has resulted from lack of interest, since attempts to obtain such information were made as early as 1954 by Zander and von Münstermann (16). These early attempts were, however, unsuccessful due to the low sensitivity of the methods available at that time.

Efforts to obtain reliable information on the concentration of progesterone in peripheral plasma and in the myometrium of pregnancy were suddenly escalated after

Csapo proposed his theory on progesterone and the defense mechanism of pregnancy (13). In spite of the tremendous advantage in sensitivity offered by the competitive protein binding and radioimmunoassay, the controversy on whether or not there is a fall in plasma progesterone level at the end of pregnancy, or before the onset of labour, is not completely resolved (see ref 63). Related to these aspects is the question of the concentration of free (unbound) progesterone in plasma, which is considered to be the biologically active form of the hormone (64). However, there have been no direct measurements of the levels of free and protein-bound progesterone in blood samples taken serially during pregnancy. The main reason for the lack of such important data is that available methods are complicated, time consuming, and far from ideal.

The demonstration of intracellular proteins that possess specific binding abilities for oestrogen and progesterone, so called receptors (26), has provided great insight into the mechanism of action of steroid hormones (see ref 18). However, there is one inherent disadvantage in data obtained from studies on receptors that limits their interpretation. These studies are almost always made on isolated systems, and since many of the receptor proteins are relatively unstable, they are carried out under somewhat artificial conditions. Consequently, we cannot be certain about the extent to which the receptor level for a particular steroid determines its concentration in the target organ. Experimental data obtained from the receptor studies have, however, provided one important lead in understanding the mechanism of action of oestrogen and progesterone. These studies have shown for example that the receptors for oestrogen and progesterone are controlled by the hormones themselves in that oestrogen has a stimulatory effect on their build up whereas, progesterone has the opposite effect (5,12,25,38,41,47). The physiological antagonism between oestrogen and progesterone is easier to understand in view of their interaction at the receptor level (5,12).

Until recently there has been some debate on the existence of specific progesterone receptors in the human uterus (e.g. 2,19,37). This question now seems to be resolved, and evidence showing that progesterone receptors are under both oestrogen and progesterone control has also been presented (12,25,38,41,47). Milgrom et al (41) reported that, after a single injection of progesterone the progesterone receptors in the guinea-pig uterus were reduced to undetectable levels within a few hours. A similar phenomenon has been shown to occur in the chick oviduct albeit somewhat different quantitatively from that reported in the guinea-pig (38). However, this baffling effect of progesterone has not yet been demonstrated in other mammalian species. Neither the biochemical mechanism nor the physiological significance of the disappearance of uterine progesterone receptors after progesterone treatment is yet clear.

From the preceding account it is obvious that in order to elucidate many aspects of steroid hormone dynamics it will be necessary to fill several gaps in the existing knowledge. Ideally, information about the following parameters should be available

before a reasonable assessment can be made of the mechanisms regulating steroid hormone concentration in tissues.

- . The total and free hormone concentration in the peripheral circulation.
- . The concentration of the hormone in the target tissue.
- . The hormone receptor level in the target tissue.

The importance of this information, particularly with regard to the endogenous steroid levels in the myometrium and decidua, has thoroughly been discussed in a recent symposium on "The fetus and birth" (Ciba Foundation Symposium 47, Elsevier, North Holland, 1977. See specially pp 427-459).

The first phase in the present investigation was concerned with the development of methods for obtaining information about the above mentioned parameters. The methods were then applied to the elucidation of mechanisms that might be responsible for regulating steroid hormone concentrations in target organs. To this end, studies were performed in both animals and man. Rabbits and guinea-pigs were chosen as experimental animals because of the marked divergence in the reproductive biology of the two species. The reproductive activity of the rabbit is acyclic in contrast to that of the guinea-pig, and progesterone withdrawal is a prerequisite for the initiation of parturition in the rabbit but not in the guinea-pig.

aim of the present study

The specific objectives of the present study can be listed as follows.

- To develop simple and reliable methods for the determination of plasma concentrations of free and protein-bound progesterone (and oestradiol) during pregnancy.
- To develop methods for the determination of oestrogen and progesterone concentrations in small samples of reproductive tissue.
- To elucidate mechanisms controlling steroid hormone concentrations in the target tissues by relating the tissue concentration of a hormone to the plasma concentration and to the concentration of hormone receptors.
- To study oestradiol and progesterone interactions in their accumulation by the target tissue.

COMMENTS ON METHODS

Determination of free and protein-bound progesterone

Since membrane filters made of cellulose esters are extremely hydrophobic, the idea was conceived that they could be used to separate free from protein-bound steroid, by adsorption of the free (non polar) steroid. This idea was tested and the experimental data showed that membrane filters (Millipore) were indeed able to adsorb large quantities of steroids from their aqueous solutions (I). In the case of progesterone (P) the adsorption of free steroid was almost 100 %. After confirming that protein-bound progesterone was not adsorbed by the filter, the method could be used to separate free from protein-bound progesterone. The reliability of the method was found to be highly satisfactory, and owing to its simplicity and rapidity of operation it was found very useful for routine analysis of free and protein-bound progesterone in blood samples. Since the filters did not adsorb all steroids as effectively as progesterone the method could not be used for the determination of either free oestradiol-17 β (E2), or testosterone or cortisol (I).

In Paper II it could be shown that data on the aqueous solubility of steroids, particularly those of more recent origin, could not be relied upon since the dissolved and undissolved fractions of the steroids were separated by membrane filters. However, by using glass fiber filters that adsorbed only insignificant quantities of the steroid from an aqueous solution, data on the solubility of E2, P and testosterone could be obtained.

In Paper III a demonstration of the use of filter method for the separation of free and protein-bound P in both competitive protein binding assay and radioimmunoassay could be given.

Determination of oestradiol and progesterone in tissue samples

After the advent of competitive protein binding and radioimmunoassays for the determination of steroids, one would have expected that data on endogenous concentrations of steroid hormones in target tissues could readily be obtained. This was not forthcoming and in our opinion, the main difficulty related to the fact that extraction of the steroid from the tissues by available methods was time consuming and yet incomplete. Since steroids in tissue are tightly bound to tissue proteins, as now confirmed by the presence of specific steroid hormone receptors, it is not surprising that the steroids could not effectively be extracted from tissues with organic solvents. Homogenization of the tissue, which itself presents difficulties in dealing with muscular tissues such as the myometrium, did not improve results greatly (VI).

The use of detergents for dissolution of proteins prior to their quantitative determination and for other related studies had already been reported (1,33,46). Taking

advantage of this information in the present study the tissue was chemically digested with a mixture of detergent and NaOH before extraction of the steroid with organic solvents (VI). In preliminary trials various combinations of NaOH and sodium dodecyl sulphate (SDS) were tested, and the one that gave most satisfactory results was used in further studies (VI). With this procedure virtually 100 % of the tissue steroids could be extracted. The organic nature of the detergent (SDS) as well as its ability for binding to protein further contributed to dislodge steroids bound to the tissue proteins. This quality of SDS, however, also resulted in its partial interaction with the steroids. The presence of SDS in the sample strongly interfered with the radioimmunoassay of the steroids, probably by degrading the antibody preparation.

Several attempts were made to remove the contaminating SDS from the extract. Procedures such as phase separation or precipitation of SDS with potassium salts or sodium perchlorate (48,59,65) were tried but met with only a degree of success. Finally, chromatography on Sephadex LH-20 was found to be satisfactory in removing the contaminating SDS from the extracted steroid hormones. This chromatographic step had in addition eliminated some of the steroids other than E2 and P but no experimental data was obtained on this point. Initially, this method was designed for the determination of P in tissue samples. Subsequent studies (VII) showed, however, that the extraction procedure was equally efficient for extracting tissue E2. The only modification needed to determine both E2 and P was to collect 6 ml rather than 3.5 ml of eluate from the Sephadex columns.

RESULTS AND DISCUSSION

The results of the investigations dealing with the development of the methods are described in Papers I, II, III, VI and VII and are commented upon in the preceding section. In this section, the data obtained from investigations in man and experimental animals will be considered and briefly commented upon.

Human studies

Free and bound progesterone in plasma during pregnancy

Plasma levels of free and protein-bound progesterone were determined in serial samples (V). It was shown that the concentration of free P like that of the total P increased steadily from week 24 to the end of pregnancy. Moreover, free P rose from 6 % of the total at week 24 to 13 % at week 40. The reason for this disproportionate increase of free progesterone after week 24 is not clear, nor is its physiological significance. The demonstration that there was no change in either total or free progesterone before our casts serious doubt on the progesterone withdrawal theory (13) for the initiation of labour in man. The data also failed to confirm a recent report by Turnbull

et al (63) showing that there is a dramatic change in the ratio of E2 to P from 15:1 at week 33 to 5:1 at week 40. The present data showed that this ratio varied little (between 6.2:1 and 7.2:1) which is in agreement with the findings of Shaaban and Kloppe (54). It is not likely that the discrepancy between our results and those of Turnbull et al (63) can be attributed to the differences in methodology, since in both instances steroid concentrations were determined by radioimmunoassay. Moreover the results of the present study have recently been confirmed by Chew and Ratnum (11). One noteworthy observation in this study pertained to the fact that the decrease in free P following labour (2 hours after) was not proportional to that in total P. Free P rose to 19 % of the total, the greatest fraction observed at any time. Two possible explanations were considered namely: 1) that the relative concentration of corticosteroids, which are capable of competing with P for binding to corticosteroid binding globulin (CBG), increased considerably; and 2) that CBG concentrations decreased significantly. Although, on the basis of available evidence, the first possibility was greatly favoured, a study was performed to obtain information on both these aspects.

The data showed (V) that not only the absolute amounts of cortisol and P but also the cortisol-progesterone ratio was important in determining the amount of P set free at any time. It was also shown that there was no change in the concentration of CBG before or during labour or until at least 5 days post partum.

Oestradiol and progesterone concentrations in reproductive tissues

In the first of this series of investigations, the concentration of E2 and P were measured in the myometrium (VIII), because it is an important target tissue for these steroids in the classical sense, and because of the well known effects of the steroids on its contractile activity.

The data showed that the tissue E2 concentration was very low relative to that in the blood or to the tissue P concentration. This was probably due to the negative influence of progesterone on E2 accumulation, in agreement with recent evidence showing that P can suppress E2 receptors (12,25). Until mid-pregnancy the P concentration was higher in tissue than in plasma, but at the end of pregnancy the ratio between the myometrial and plasma concentrations had decreased to about 0.6. This suggests that the binding sites for P, specific as well as non specific, are saturated as pregnancy approaches full term.

One interesting finding, that remains to be explained, is that the E2 concentration in myometrial tissue was significantly influenced by the proximity to the placenta, whereas P concentration was not. The lack of influence of the placenta on the myometrial P concentration argues against the concept of a local "block" of myometrial activity by a high P concentration at the placental site (14). The data are in agreement with those reported by Runnebaum and Zander (50), who showed that the dif-

ence in P concentration at the placental and antiplacental sites was not significant at full term. Since the myometrial E2 concentration was significantly influenced by the localization of the placenta, it might be tempting to suggest that the high E2 concentration at the placental site could act as a local "block" for the myometrial activity. This line of argument is weakened by the finding that the myometrial E2 concentration increased little in pregnancy, but further investigation is needed to completely rule out the possibility.

The concentrations of E2 and P were also examined in the decidual and placental tissues (Paper IX). No other data are available on the concentration of E2 in the placenta or of either steroid in the decidua (16,51).

The concentration of E2 in the decidua was also very low and not significantly different from that in the myometrium. However, the decidual protein content per unit weight was lower than that of the myometrium particularly at mid-term and, in contrast to that of the myometrium, it increased at term. Thus the differences between myometrium and decidua with respect to their steroid concentrations depend to some extent on the mode of expression (wet weight or protein). At full term the decidual E2 concentration (per g wet wt) was lower than that of the myometrium and the ratio between the tissue and plasma concentrations was only 0.08 for the decidua, whereas it was 0.12 for the myometrium. This indicates that decidua has an extremely low capacity for E2 accumulation, which might result from P influence on E2 binding in tissue, as previously suggested for the myometrium (12,25). The present data on P concentrations in these tissues support this suggestion, since the P concentration in the decidua nearly doubled from mid-term to term, whereas in myometrium the increase was not significant.

In several recent theories concerning mechanisms for the initiation of human parturition, decidual E2 and P were assigned a key role in the production of prostaglandins, which in turn led to the expulsive myometrial contractions (10,20,34). Oestrogens and P, in these proposals, are assumed to control the stability of decidual lysosomes (see also 29,52,60).

The relatively low decidual and myometrial E2 concentrations in pregnancy, and the observation of only minor changes towards term might be taken as an argument against a direct or dominant role for this steroid in the onset of labour. On the other hand, the high concentration of P in the decidua, increasing significantly at term, might be considered as an argument for its role in the control of parturition. However, P is assumed to stabilize decidual or foetal membrane lysosomes, whereas the proposed mechanism for the increased production of prostaglandins is labilization of the lysosomes (20,34,39,52). Milewich *et al* (39) have recently proposed that altered metabolism and binding to a specific protein of P in foetal membranes (53) reduces the foetal concentration of progesterone that is available to act as a stabilizer for foetal membrane lysosomes. It would be interesting to know the endogenous levels of E2

and P in foetal membranes, how they alter with advancing pregnancy, and what is their relation to the concentrations in the decidua and myometrium. In this connection, it may be relevant to recall that P-E2 ratio in the myometrium decreased at term (IX), particularly since recent studies (unpublished) indicate that this ratio is lower in the myometrium of women in labour than of those not in labour.

The concentration of P in the placenta was already very high at mid-term and did not show any further increase at term, which is in agreement with the recent data of Runnebaum et al (51). The placental E2 concentration, on the other hand, was unexpectedly low at mid-term and increased significantly towards term. The increase in placental E2 concentration from mid-term to term might reflect a foetal contribution. This view seems logical since the adrenals of the foetus are not fully developed until around mid-term and therefore the foetal supply of E2 precursors is limited. This is also compatible with the existing evidence that whereas placental production of P is more or less independent of the foetus, E2 production during pregnancy is to a great extent dependent on the supply of precursors from the foetus.

The results of these studies provide further evidence for a poor reflection of plasma steroid levels in target tissues such as the myometrium and decidua. It is also noteworthy that the decidua and myometrium, although anatomically related, differ in their ability to accumulate steroids. This difference may be related to their functional characteristics. The function of the decidua in parturition is far from clear (9,34,53), whereas that of the myometrium is very evident.

The last study on human reproductive tissue concerned the determination of E2 and P concentrations in the Fallopian tube, and again included an analysis of the relationship between plasma and tissue concentrations (X). Studies were made in non-pregnant women at three different stages of the menstrual cycle and steroid concentrations were determined in both the ampullary and the isthmus regions of the tube. No previous data is available on the concentration of either E2 and P in the human Fallopian tube.

Although the concentration of both E2 and P was always slightly higher in the ampulla than in the isthmus, the difference was not statistically significant in any of the three phases examined, namely the follicular, ovulatory and luteal phases of the menstrual cycle.

The protein concentration per unit weight was considerably higher in the isthmus, which is more muscular tissue than the ampulla. In fact, the protein content in the isthmus (160 mg/g wet wt) compared rather well with that found in the myometrium (140 mg/g wet wt). There was, however, no significant variation in the protein content of either the ampulla or the isthmus between the various phases. This justifies a comparison of steroid levels in different phases in the same tissue.

In general, the correlation between the tissue and plasma concentrations was poor and there was always a considerably higher steroid level in the tissue than in the

asma. This suggests that the Fallopian tube may be considered as a target organ for male sex steroids. Indeed, the existence of E2 and P receptors has recently been demonstrated in the Fallopian tube (43,49). Since the Fallopian tube in contrast to the uterus, does not exhibit proliferative and/or secretory changes following hormonal treatment the precise action of E2 and P on the tube cannot be stated. These hormones do, however, seem to modify tubal motility (see ref 17) although the mechanisms by which this is achieved remain unknown.

2. Studies on experimental animals

Rabbits

Previous studies in the rabbit have shown that changes in uterine progesterone during pregnancy (9) and pseudopregnancy (62) do not follow those in plasma progesterone. It is therefore of interest to study the relationship between plasma and uterine E2 concentrations particularly in view of evidence that plasma E2, unlike plasma P, changes little during pregnancy (8,24). The data (Paper XI) showed that uterine E2 changed considerably during pseudopregnancy in the rabbit, in spite of relatively constant level in the plasma. The changes in tissue E2 were therefore analysed in relation to the changes in both plasma P and uterine P reported previously (62). The results of this analysis showed that the most important factor controlling tissue E2 was neither plasma E2 nor plasma P but probably the tissue concentration of P. These results are consistent with the finding that P causes a reduction of E2 receptors in the uterus (12,25).

During the course of these and other studies in rabbits (4,31) we observed a great variation in the plasma concentration of E2 in the control (non-pregnant) group, in addition to which a significant degree of failure in the maintenance of pseudopregnancy was met with in some rabbits (unpublished observations). In separate studies we also observed a considerable variation in the in vivo myometrial activity of the so-called oestrous rabbits (4,30). These observations together with the early data (1,23) showing that in the rabbit ovary, mature follicles do not survive indefinitely but regress within 7 to 10 days, led us to examine the variation in plasma E2 levels that occurred in the same rabbit on different days (XII).

The data revealed that there was a considerable variation in plasma E2 concentration from one sampling day to another in the same rabbit. The variations were similar in the three different seasons, namely January, April and September, in which the rabbits were studied. There was, however, a significant difference between the mean values for plasma E2 obtained in January (33.6 pg/ml), April (29.9 pg/ml) and September (22.4 pg/ml). The pattern of variation in E2 levels in individual rabbits tended to be cyclic, and this cycle was roughly of the order of eight days. However, due to the limited number of observations, the evidence for the cyclicity in the plasma E2 levels of non-pregnant rabbit cannot be considered as yet definitive. It

is interesting to note, however, that in the studies by Smelser *et al* (57) and Hill and White (23) the cycle of follicle growth and decline was of the order of 7-10 days. The occasional failure in maintaining pseudopregnancy (HCG or LH induced) in rabbits might therefore be explained on the basis of E2 levels that are too low to maintain a corpus luteum. Although ovulation will occur, particularly when induced by the exogenous administration of HCG or LH, there is evidence that a certain level of E2 is required to maintain the corpus luteum (42,58).

Interestingly no relationship between the appearance of the external genitalia (recorded on each sampling day) and the measured E2 levels in plasma could be found in the present study. From this observation a case can be made for the redefinition of an oestrous rabbit. Furthermore, the data on the variation of plasma E2 concentration in the non-pregnant rabbit can be of considerable importance in designing endocrinological experiments in rabbits where the influence of E2 is suspected to be an important factor.

In order to further elucidate interactions between E2 and P in the uterus, the effect of P and its subsequent withdrawal was investigated during a period when high and steady levels of plasma E2 were maintained (XIII). Preliminary data had shown that relatively constant levels of E2 could be obtained by a single injection of Estradurin (poly-oestradiol phosphate). The administration of P did not influence E2 levels in the plasma. However, the tissue E2 concentration showed a significant decrease after four daily injections of P. When P injections were halted for two days (progesterone withdrawal) tissue E2 levels rose and were greater than those found in rabbits that did not receive P. Thus it could be demonstrated that the tissue E2 concentration could be elevated merely by withdrawing the inhibitory influence of P while keeping plasma E2 levels constant. This rebound effect of P on tissue E2, which has not been demonstrated previously, may be of physiological significance. Furthermore the data provide evidence for oestrogen-progesterone antagonism even under experimental conditions.

A great deal of the data obtained on E2 and P concentrations in the rabbit uterus could be interpreted by a consideration of the effects of P on P and E2 receptors. In general, P has been shown to lower both E2 and P receptors in the uterus (5,12,25). However, in mammals the decay of P receptors following P administration has been demonstrated only in the guinea-pig (41). Recently, a similar phenomenon was reported to occur in the chick oviduct and some quantitative differences from the guinea-pig were noted (38). It was therefore important to find out whether a P-induced reduction of P receptors occurred in the rabbit and, if it did, to what extent it was quantitatively comparable to that reported in the guinea-pig.

Neither the biochemical mechanism nor the physiological significance of the disappearance of uterine P receptors after P treatment is known. It was therefore hoped that, by studying the subcellular distribution of P and E2 before and after P treat-

INTERRELATIONS BETWEEN PLASMA AND TISSUE CONCENTRATIONS OF
17 β -OESTRADIOL AND PROGESTERONE DURING HUMAN PREGNANCY

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SUMMARY

Oestradiol and progesterone concentration in plasma, decidua, myometrium and placenta obtained from women undergoing Cesarean section at term and abortion at 16-22nd weeks of pregnancy were determined. There was a significant increase in oestradiol concentration (per g wet wt) both in placenta, decidua and myometrium from mid-term to term. Both at mid-term and term oestradiol concentrations in decidua and myometrium were much smaller than those in the plasma (per ml).

Progesterone concentration in placenta and in myometrium did not increase from mid-term to term whereas it increased significantly in decidua. Decidual and myometrial progesterone concentrations at mid-term were 2-3 times higher than those in plasma, but at term the concentrations in both these tissues were lower than in plasma. The ratio progesterone/oestradiol in plasma, decidua, myometrium and placenta at mid-term was 8.7, 112.2, 61.4 and 370.0 respectively and it decreased significantly in the myometrium and placenta but was nearly unchanged in plasma and decidua at term.

The general conclusion to be drawn from the present study is the lack of correspondence between the plasma concentrations and the tissue concentrations of female sex steroids during pregnancy.

Data of previous studies have shown that the concentration of 17 β -oestradiol and progesterone, in the myometrium does not correspond to the concentrations found in the plasma at the end of pregnancy (Batra & Bengtsson, 1978). Another interesting finding in that study was that the capacity of the myometrium for oestradiol in contrast to progesterone accumulation was relatively little. The myometrium/plasma ratio for oestradiol at the end of pregnancy was less than 0.2. Furthermore, the local influence of placenta was significant in determining oestradiol concentration but not progesterone concentration in the myometrium.

In several recent theories concerning mechanisms for the initiation of human parturition, decidual oestradiol and progesterone was assigned in key role in the production of prostaglandins which in turn led to the expulsive myometrial contractions (Liggins, 1974; Gustavii, 1975; Challis *et al.*, 1977). Estrogens and progesterone in these proposals were assumed to control the stability of decidual lysosomes (see also Schwarz *et al.*, 1975; Sykes *et al.*, 1975; Kierse & Turnbull, 1976). We wondered therefore whether the concentrations of these steroids in the decidua were related to those in the plasma and/or those in the myometrium, and if so in what manner.

In the present study we obtained data on the concentration of oestradiol and progesterone in samples of plasma, myometrium, decidua as well as placenta at mid-term and at full term of pregnancy. The data were analysed in terms of the interrelationships between the concentration of either steroid in various ^{the} tissues and in plasma as well as the extent to which these relations are altered from mid-term to the full term of pregnancy.

MATERIALS AND METHODS

Tissue and blood samples. Samples of myometrium, decidua and placenta were obtained from 13 healthy women at elective Cesarean sections performed in 38-42 weeks of pregnancy and collected in ice-cold Krebs-Ringer solution as described previously (Batra & Bengtsson, 1976). Similarly samples were also obtained from 9 patients in the 16th-22nd week of pregnancy undergoing abortion by hysterotomy. The tissue pieces were thoroughly rinsed in the Krebs-Ringer medium and samples of myometrium and placenta weighing 200-400 mg whereas the whole amount of decidua obtained were blotted dry, weighed and frozen at -20°C , until used for analysis. There is no significant loss of either steroid from any of the tissues when they are left at 4°C for 1 hour. However, steroids are lost from the tissue with incubation at room temperature (Batra, 1976).

From each patient venous blood (about 10 ml) was collected in heparinized glass tubes, centrifuged and the plasma stored frozen at -20°C until analysed.

Tissue digestion and extraction of steroids. The frozen tissues were thawed and then digested in 0.5 ml mixture containing 5% sodium dodecyl sulfate (SDS) and 0.5 N NaOH as described previously (Batra & Bengtsson, 1976). The digested material was extracted three times with 3 vol ethyl acetate. The combined extracts were evaporated to dryness under air at 40°C . The dried extract, however, contained a significant amount of SDS which was removed before radioimmunoassay (RIA) by Sephadex LH-20 chromatography, and 6 ml eluate were collected (Batra & Bengtsson, 1976, Batra, 1976).

Radioimmunoassay (RIA). After evaporation of the solvent, the residue from the eluate was dissolved in 1 ml ethyl acetate, and a suitable amount depending on the predicted concentration of

Oestradiol and progesterone was taken for the respective RIA. The procedure for RIA of oestradiol was that described by Lindberg *et al.* (1974). The reliability of this assay for determination of oestradiol in tissue extracts has previously been checked by us (Batra, 1976). The recovery of authentic unlabelled oestradiol added to digested samples and calculated at the end of the completed procedure was 88.5% (Batra, 1976). The coefficient of variation in replicate analysis of oestradiol was about 10%.

The method for progesterone determination was essentially similar to that described by Youssefnejadian *et al.* (1972). The antiserum (FO 22.5.73), which was a gift from Dr Kjell Martinsson (Royal College of Veterinary Medicine, Stockholm) and found to be highly specific for progesterone, was used in a dilution of 1/1500 (vol/vol). Other details have been described previously (Batra & Bengtsson, 1976). There was an almost complete recovery of not only radiolabelled progesterone, but also of unlabelled progesterone (96.5%) added to digested tissue and determined by RIA after extraction (Batra & Bengtsson, 1976). Also oestradiol and progesterone concentrations in plasma were determined by these radioimmunoassays.

Protein concentration in the digested tissue was determined by the method of Lowry *et al.* (1951) by using bovine serum albumin, dissolved in digestion mixture, as standard.

Chemicals. Sodium dodecyl sulfate (SDS) was purchased from Sigma Chemical Co. Oestradiol and progesterone were purchased from Ikapharm Israel. Radioactive 2,4,6,7- H^3 oestradiol (102 Ci/mmol) and 1,2,6,7- H^3 progesterone (105 Ci/mmol) were obtained from New England Nuclear Corporation, and Sephadex LH-20 from Pharmacia Fine Chemicals Sweden.

Statistics. The mean values from different groups were compared according to Student's t test.

RESULTS

Fig. 1 shows the mean concentrations of oestradiol in placenta, decidua, myometrium (per g wet weight) and in plasma (per ml) from pregnant women. Plasma oestradiol increased 4-fold from mid-term to term whereas the placenta oestradiol just about doubled. There was also a significant increase in the oestradiol concentration in both myometrium and decidua. At mid-term there was no significant difference between oestradiol concentration in decidua and myometrium and concentrations in both these tissues were much smaller than that in the plasma.

In the case of progesterone there were some important differences in these interrelationships as shown in Fig. 2. There was a similar increase in progesterone concentration in the plasma as seen for oestradiol concentrations. However, the concentration of progesterone in placenta did not increase from mid-term to term which is in contrast to oestradiol concentrations (Fig. 1, but see also Fig. 4). The concentration of progesterone in the decidua and myometrium at mid-term were 2-3 times higher than the concentration in plasma. At term, however, concentrations of progesterone in neither of these tissues exceeded plasma concentration. Whereas the increase in decidual progesterone concentrations from mid-term to term was significant, it was not for myometrial progesterone.

The quantitative relationships between plasma and the tissue concentrations of either steroid are shown in Table 1. These data

show that the capacity of decidua and myometrium for oestradiol accumulation is very low. Due to a large increase in plasma oestradiol concentration and relatively little increase in the tissue concentrations as the pregnancy advanced from mid-term to term, the tissue/plasma ratio in the case of both decidua and myometrium is further reduced. The concentration of progesterone in decidua and myometrium at mid-term was 3.5 and 1.9 times respectively higher than that in the plasma (Table 1). However there was a significant reduction in these ratios when the pregnancy advanced from mid-term to term.

Table 2 shows the relative concentration of progesterone and oestradiol in plasma and in the tissues at both mid-term and term. Plasma concentration of progesterone is about 8 times higher than oestradiol at both mid-term and term. Although the change in the relative concentration of steroids was insignificant in decidua it was significant in myometrium. The progesterone/oestradiol ratio in placenta was about halved from mid-term to term and this reduction was statistically significant.

When steroid concentrations in the tissues were calculated on the basis of protein content it was found that although the general pattern was similar to that shown in Fig. 1 and 2 (per gram wet weight), the quantitative relationships were somewhat changed. This was due to the difference in protein concentrations per unit weight in different tissues (Fig. 3). Firstly, the protein concentration in the myometrium at mid-term was significantly higher than that in the decidua or placenta. Secondly, whereas the protein concentrations in both placenta and decidua increased significantly from mid-term to term, that in the myometrium remained virtually unchanged. This resulted in the fact that at term the difference

between protein concentrations in myometrium and decidua was no longer significant but the difference in myometrium and placenta was still significant.

When oestradiol concentrations were expressed in terms of protein (Fig. 4), the myometrial oestradiol concentration decreased in relation to placental and decidual concentrations. This was most marked at mid-term. Now, oestradiol concentration in the myometrium was lower than that in the decidua (compare with Fig. 1). Furthermore, the difference between placental concentrations at mid-term and term became less significant and that between decidual concentrations became insignificant (compare with Fig. 1).

In the case of progesterone (Fig. 5) similar change occurred but no important difference in the quantitative relationships between different tissues was revealed (compare with Fig. 2).

DISCUSSION

There has been a general trend while studying the effects of estrogens and progesterone on various female reproductive tissues to relate the intensity of the effect to the concentration of hormones in the blood. It is, however, more reasonable to think that the biological activity will perhaps be governed by concentrations in the tissue. A recent study from this laboratory showed that the concentration of oestradiol and progesterone in the myometrium of pregnancy did not reflect the concentration found in the plasma (Batra & Bengtsson, 1978). The results of the present study show that the decidual concentration of oestradiol, as that of the myometrium, during pregnancy is very low as compared to that of plasma

(Fig. 1). In fact at full term, decidual oestradiol concentration was lower than the myometrial concentration indicating extremely low capacity of decidua for oestradiol accumulation. This might be the result of progesterone influence on oestradiol binding in the tissue as previously suggested for the myometrium (Batra & Bengtsson, 1978). The data on progesterone concentrations in these tissues support this suggestion. Whereas the progesterone concentration in the decidua from mid-term to term nearly doubled, the increase in myometrium was not significant (Fig. 2). There are no other data published on the concentrations of these steroids in the decidua. The data on the concentrations in the myometrium are in agreement with other available data (Runnebaum & Zander, 1971; Batra & Bengtsson, 1978; Ferré et al., 1978).

The relatively low decidual and myometrial ~~o~~estradiol concentration in pregnancy and the indication of only minor changes towards term might be taken as an argument against a direct or dominant role of this steroid in the onset of labour. On the other hand the high concentration of progesterone in the decidua that increased significantly at term might be considered to support the argument for its role in the control of parturition. However, progesterone is assumed to stabilize decidual, or fetal membrane, lysosomes which in contrast with the proposed mechanism for the increased production of prostaglandins resulting from the labilization of the lysosomes (Gustavii, 1975; Schwarz et al., 1975). Milewich et al. (1977) have recently proposed that altered metabolism and binding to a specific protein of progesterone in fetal membrane (Schwarz et al., 1976) reduces the local concentration of progesterone that is available to act as a stabilizer on fetal-membrane

lysosomes. It would be interesting to know the endogenous levels of oestradiol and progesterone in fetal membranes, how they alter with advancing pregnancy and what is their relation to the concentrations in the decidua and myometrium. In this connection, the results showing that progesterone/oestradiol ratio in the myometrium (Table 2) decreased at term may be of importance in view of the fact that recent studies (unpublished) indicate that this ratio was higher in myometria of women not in labour compared to those in labour. The myometrial activity in these women was recorded before a Caesarean section performed at term.

The concentration of progesterone in the placenta was very high already at mid-term and did not show further increase at term (Fig. 2). This is in agreement with recent data of Runnebaum et al. (1975). The placental oestradiol concentration was unexpectedly low at mid-term compared to progesterone concentration (Figs 1 and 2). However, in contrast to progesterone it increased significantly towards term (Fig. 1). This increase in placental oestradiol concentration might reflect fetal contribution. This seems logical in view of the fact that until around mid-term adrenals of the fetus are not fully developed and therefore the fetal supply of oestradiol precursors is limited. This is also compatible with the existing evidence that the production of progesterone by the placenta is more or less independent of the fetus whereas oestradiol production during pregnancy is to a great extent dependent on the supply of precursors from the fetus.

Although the general relationship between the steroid concentrations in various tissues were well maintained when expressed in terms of their protein content (Figs 4 and 5), it is of interest that the protein concentrations per unit weight can greatly vary between different tissues (Fig. 3). It was, however, not surprising

to find that the myometrium, which is a muscular tissue, had higher protein content than tissues devoid of muscle as placenta and decidua. The finding that the protein concentration in placenta and decidua, in contrast to myometrium, increased from mid-term to term is somewhat puzzling. One explanation for this might be that hypertrophy exceeds hyperplasia under the continuous influence of oestradiol and progesterone. Another possibility might be that there is a loss of fluid in these tissues after mid-term. This later explanation is consistent with the general observation that placenta in early pregnancy is less solid than at term.

The results of the present study provide further evidence for a poor reflection of plasma concentrations of steroids in the tissues during pregnancy. Furthermore, the capacity of not only the myometrium but also of decidua for oestradiol in contrast to progesterone accumulation was very low.

ACKNOWLEDGEMENTS

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Table 1. Quantitative relationships between tissue and plasma concentrations of oestradiol and progesterone at mid-term and term

Pregnancy length	Decidua/plasma		Myometrium/plasma	
	Oestradiol	Progesterone	Oestradiol	Progesterone
Mid-term n = 9	0.1 ± 0.4	3.48 ± 1.43	0.17 ± 0.03	1.88 ± 0.30
Term n = 13	0.08 ± 0.01 ^{xxx}	1.03 ± 0.12 ^x	0.12 ± 0.01	0.47 ± 0.04 ^{xxx}

Values are mean ± S.E., ^x P < 0.05, ^{xxx} P < 0.005.

Table 2. Relative concentrations of progesterone and oestradiol (progesterone/oestradiol) in different tissues at mid-term and term.

Pregnancy length	Plasma	Decidua	Myometrium	Placenta
Mid-term n = 9	8.7 ± 1.3	112.2 ± 20.2	61.4 ± 11.4	370.0 ± 43.4
Term n = 13	8.7 ± 0.6	124.3 ± 15.2	36.1 ± 3.4 ^x	187.7 ± 20.3 ^{xxx}

Values are mean ± S.E., ^x P < 0.05, ^{xxx} P < 0.005.

Legends to figures

- Fig. 1 Oestradiol concentrations (mean $\pm$ S.E.) in plasma, myometrium decidua and placenta at mid-term (n = 8) and term (n = 13), ^{xxx} P < 0.005.
- Fig. 2 Progesterone concentrations (mean $\pm$ S.E.) in plasma, myometrium, decidua and placenta at mid-term (n = 8) and term (n = 13), ^x P < 0.05.
- Fig. 3 Protein content per g wet weight (mean $\pm$ S.E.) in different tissues at mid-term (n = 9) and term (n = 13), ^{xxx} P < 0.005.
- Fig. 4 Oestradiol concentrations (mean $\pm$ S.E.) in different tissues expressed on the basis of protein content, ^x P < 0.05, ^{xxx} P < 0.005.
- Fig. 5 Progesterone concentrations (mean $\pm$ S.E.) in different tissues expressed on the basis of protein content.

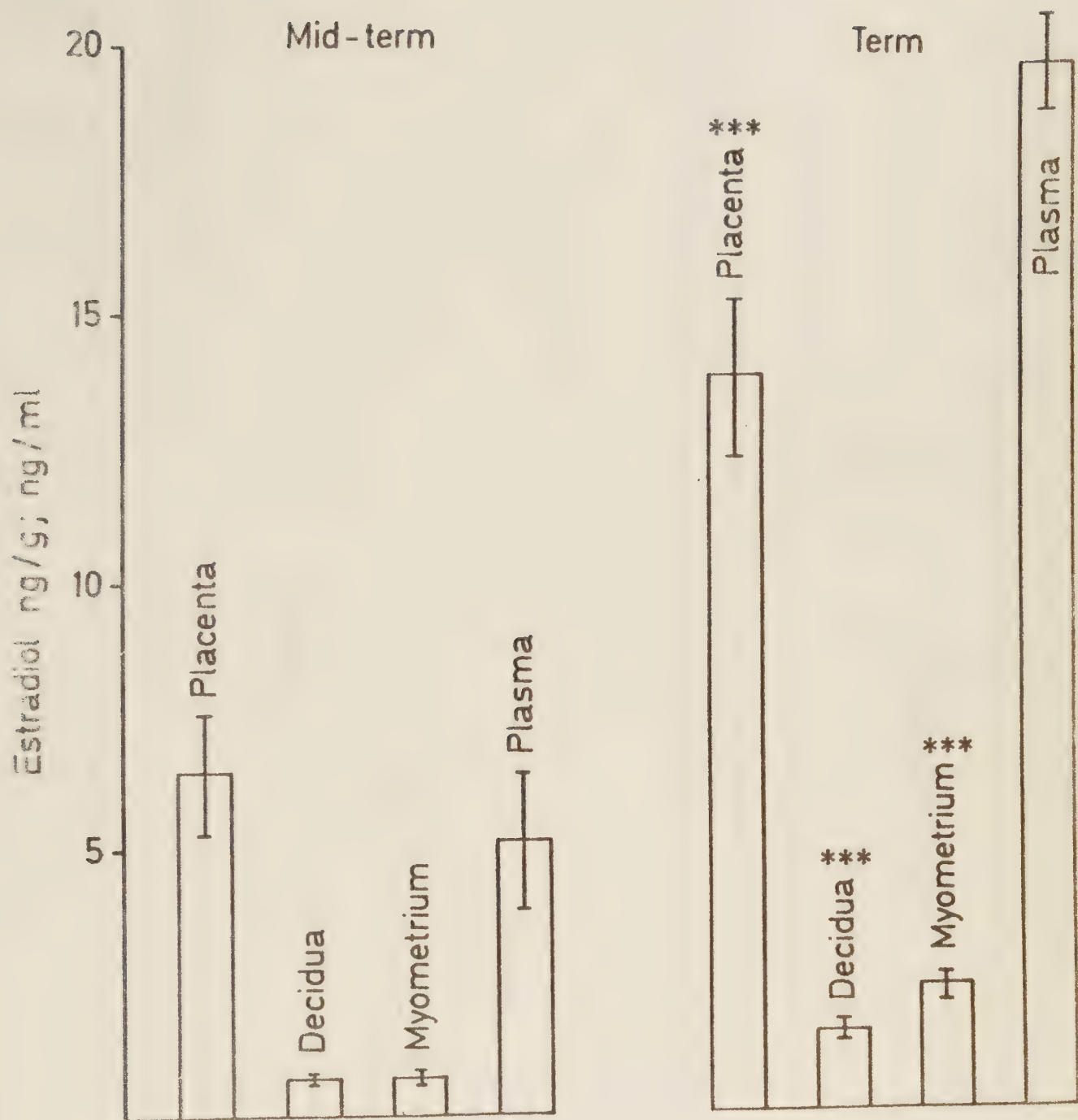


Fig.1

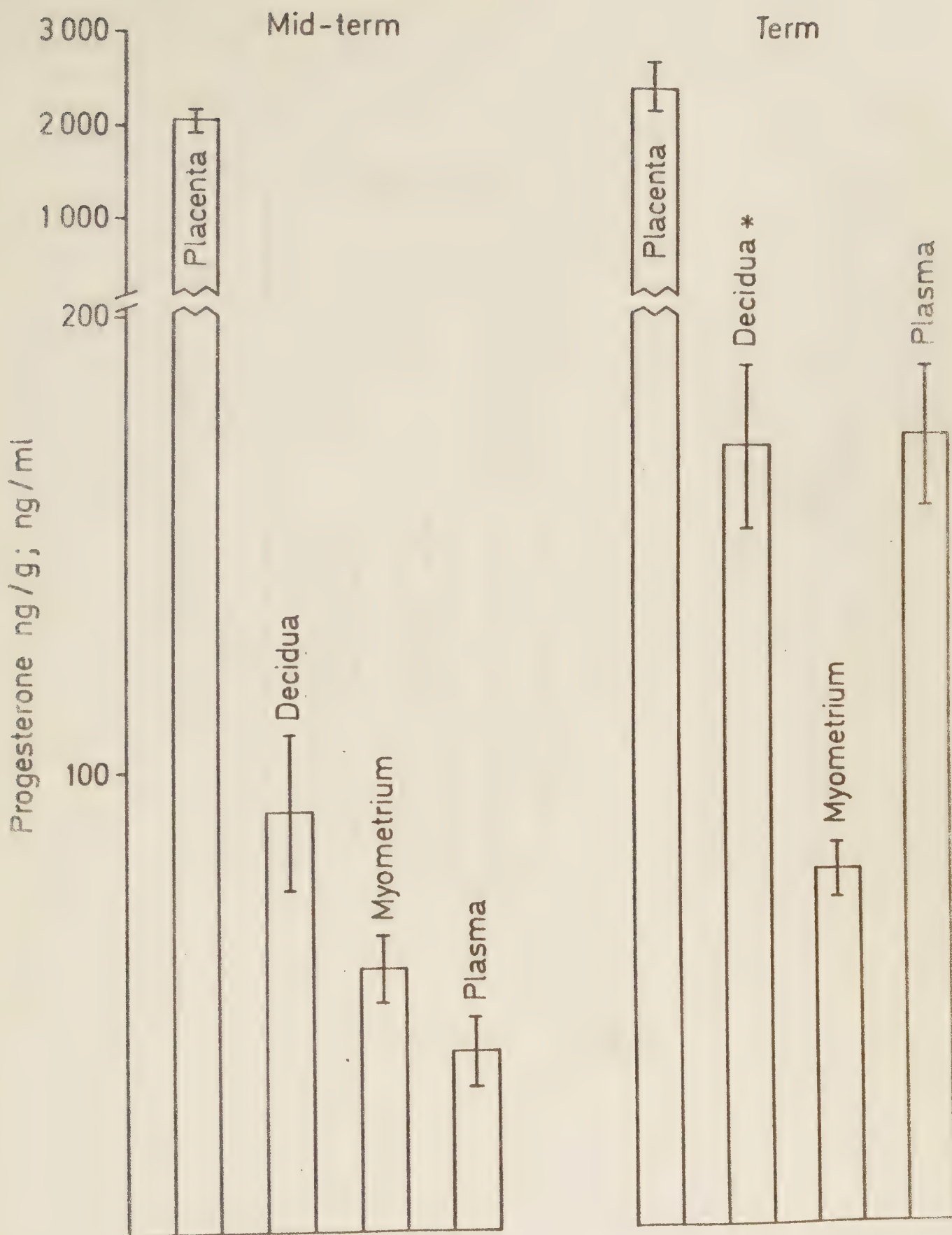


Fig.2

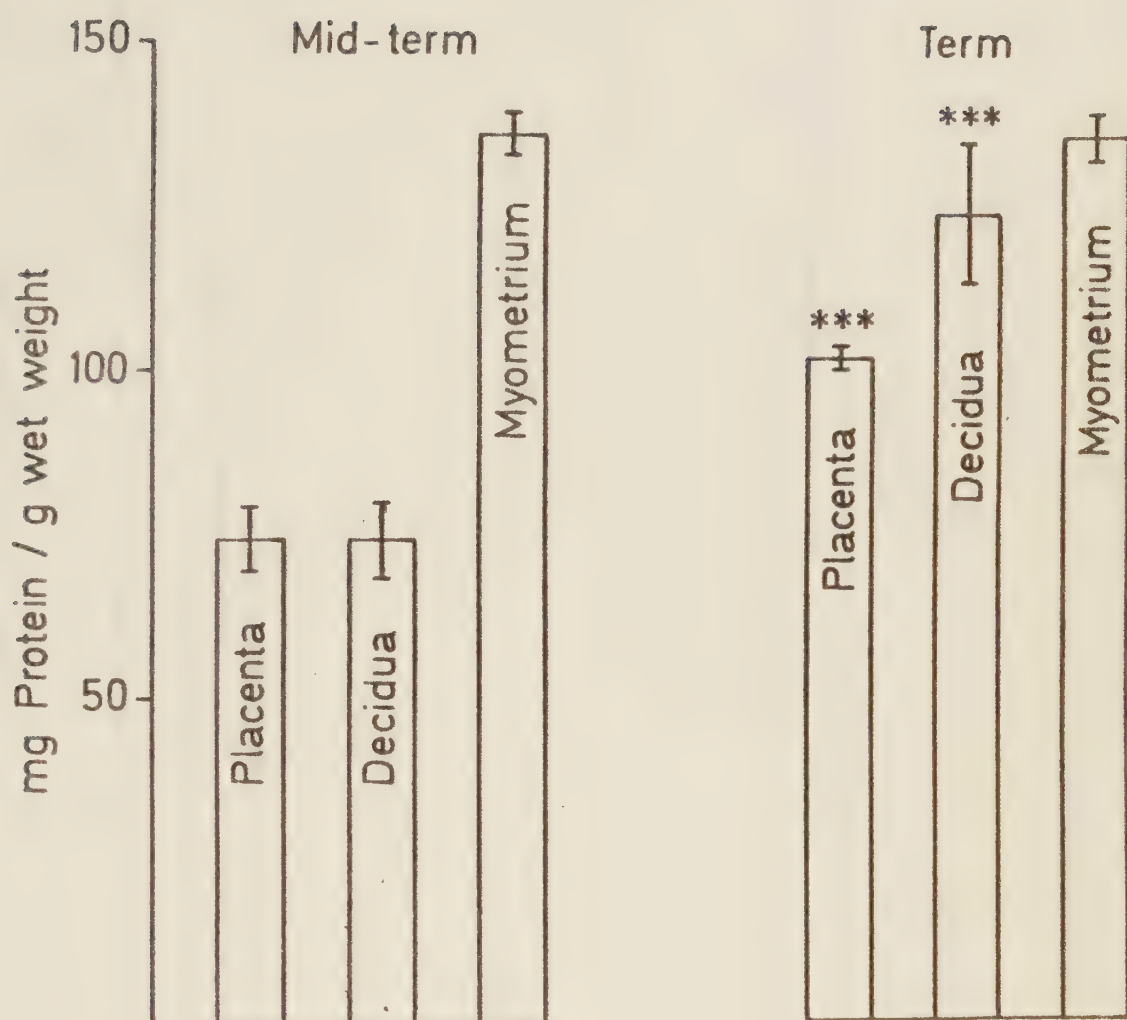


Fig.3

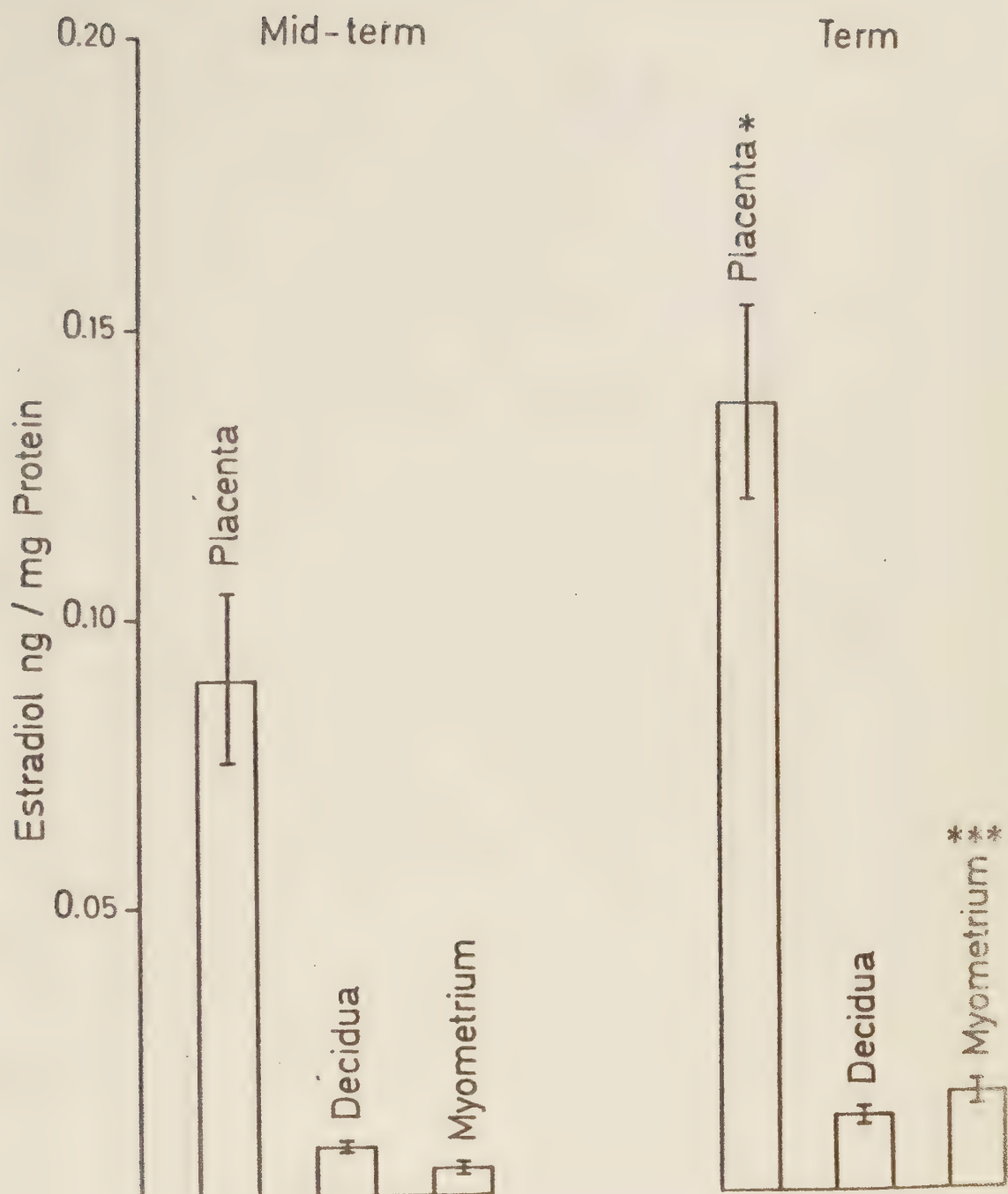
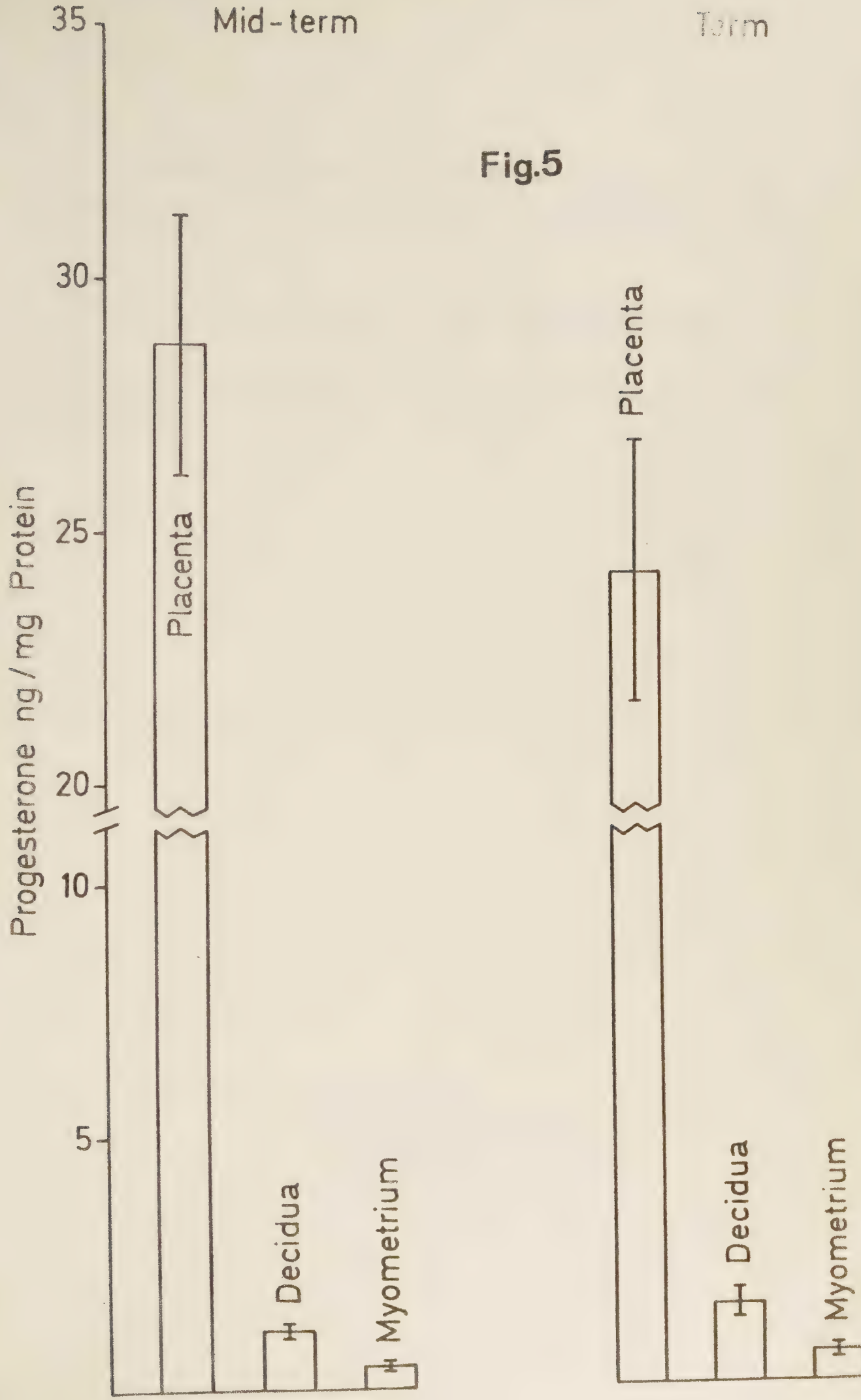


Fig.4



Female Sex Steroid Concentrations in the Ampullary and Isthmic Regions of the Human Fallopian Tube and their Relationship to the Plasma Concentrations During the Menstrual Cycle

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ABSTRACT. The concentrations of estradiol-17 β (E₂) and progesterone (P) were measured in the ampullary and isthmic portion of the Fallopian tube of non-pregnant menstruating women and the cyclic fluctuations related to the concentrations of these hormones in plasma. The steroid concentrations were determined by radioimmunoassays. Due to the marked difference in the protein concentrations of the two regions, the steroid concentrations were measured not only on the basis of wet weight but also of protein content. There was no significant difference in the isthmic and ampullary concentrations of either steroid in any of the menstrual phases. The mean value for E₂ was highest in the ovulatory phase and for P during the luteal phase. The tissue (per g)/plasma (per ml) ratio for the steroid concentrations was above unity in all measurements. The ratio for E₂ was highest (isthmus: 12, ampulla: 8) in the follicular phase and for P (isthmus: 26, ampulla: 18) during ovulation. Since these highest ratios were attained when plasma steroid concentrations were relatively low they were interpreted as reflections of a maximal receptor contribution.

Introduction

GENERALLY, it is not known how changes in plasma estrogen and progesterone levels during menstrual cycle are reflected in their target organs. Recent data on the concentration of these hormones in the endometrium and myometrium indicate that there is an overall poor correlation between plasma and tissue levels.¹⁻³ There is as yet no data available on the concentrations of these hormones in the Fallopian tube of the human or experimental animals. Such data are important not only from the point of view of steroid dynamics in this organ, but also with regard to the fact that estrogen and progesterone govern the motor activity of the tube and thereby probably also the transport of the ovum towards the uterus (see Ref. 4). Steroid-sensitive adrenergic nerves and adrenoceptors in the tube is one of the mechanisms which mediates the pattern of tubal mobility during the menstrual cycle.^{5, 6} In the present study the concentrations of estrogen and progesterone were determined in the ampullary and isthmic regions of the tube during the follicular, ovulatory, and luteal phases of the cycle of menstruating women and correlated with the steroid concentrations in plasma.

Material and Methods

Material

The Fallopian tube from one or both sides were obtained from 33 menstruating women (31 - 53 years) subjected to abdominal hysterectomy because of adenomyosis or myoma, or salpingo-oophorectomy for benign ovarian cysts. Material from patients receiving hormone treatment in one form or another was not included.

Morphine chloride (10 mg) and scopolamine bromide (0.4 mg) were given as pre-anesthetic medication. Anesthesia was induced by pentothal sodium administered intravenously, followed by inhalation of a mixture of dinitrous oxide and oxygen together with pethidine chloride intravenously. Succinylcholine iodide was given initially as muscle relaxant, followed by pancuronium bromide.

Immediately after removal of the tubes, surrounding connective tissue was dissected away from the muscle wall. A thin (2 mm o.d.) probe was introduced into the lumen from the ampullary end in order to localize the ampullary-isthmic junction, where the oviduct was divided. A few mm of the adjacent isthmic and ampullary portions, as well as the infundibular part of the tube, were discarded. The remainder was lightly blotted on filter paper, weighed, and stored frozen (-18°C) until analyzed.

Venous blood (approximately 10 ml) was drawn from each woman during the operation

into heparinized tubes and centrifuged. The plasma was stored frozen at -18°C .

Tissue digestion and extraction of steroids

The frozen tissues were thawed and then digested in a 0.5 ml mixture containing 5 % sodium dodecyl sulfate (SDS) and 0.5 N NaOH as described previously.⁷ The digested material was extracted three times with 3 vol. ethyl acetate. The combined extracts were evaporated to dryness under air at $+40^{\circ}\text{C}$. The dried extract, however, contained a significant amount of SDS, which was removed before radioimmunoassay (RIA) by Sephadex LH-20 chromatography, and 6 ml eluate were collected.^{7, 8}

Radioimmunoassay

After evaporation of the collected eluate, the residue was dissolved in 1 ml ethyl acetate and a suitable amount, depending on the predicted concentration, of 17β -estradiol (E_2) and progesterone (P) was taken for the respective RIA.

The procedure for RIA of E_2 was that described by Lindberg *et al.*⁹ The reliability of this assay for determination of E_2 in tissue extracts has previously been checked.⁸ The recovery of authentic unlabelled E_2 added to digested samples and calculated at the end of the completed procedure was 88.5 %.⁸ The coefficient of variation in replicate analysis of E_2 was about 10 %.

The method for P determination was essentially similar to that described by Youssefnejadian *et al.*¹⁰ The antiserum (FO 22.5.73), which was a gift from Dr. Kjell Martinsson (Royal College of Veterinary Medicine, Stockholm) and found to be highly specific for P, was used in a dilution of 1/1500 (vol/vol). Other details have been described previously.⁷ There was an almost complete recovery of not only radiolabelled P, but also of unlabelled P (96.5 %) added to digested tissue and determined by RIA after extraction.⁷

Protein concentration in the digested tissue was determined in the tissue by Lowry *et al.*¹¹ using bovine serum albumin, dissolved in digestion mixture as standard.

Also, E_2 and P concentrations in plasma were determined by these radioimmunoassays.

Definition of cycle stage

The material was divided into three groups with regard to the phase of the menstrual cycle on the basis of plasma steroid levels. The follicular phase included women with plasma $P \leq 2$ ng/ml and $E_2 \leq 100$ pg/ml, the ovulatory phase women with the same plasma P levels, but $E_2 > 100$ pg/ml, and the luteal phase, those having $P > 2$ ng/ml. The mean values for the plasma steroid levels in the three groups are illustrated in Fig. 1. Whenever material was available the cyclic stage was confirmed on hematoxyline-eosin stained endometrial biopsies. The mean ages of the women in the groups thus defined, showed no statistically significant difference from one another.

Chemicals

SDS was purchased from Sigma Chemicals Co.. E_2 and P were purchased from Ikapharm, Israel. Radioactive $[2, 4, 6, 7, - H^3]$ estradiol (102 Ci/mmol) and $[1, 2, 6, 7 - H^3]$ progesterone (105 Ci/mmol) were obtained from New England Nuclear Corporation, and Sephadex LH-20 from Pharmacia Fine Chemicals, Sweden.

Statistics

Mean values for the steroid and protein concentrations in the various groups were compared using the unpaired Student's t -test, and the levels in the ampulla and isthmus and left and right tubes, respectively, compared in the paired t -test. Linear regression analysis was done with the least square method.

Results

There was a marked difference in the protein content per unit weight in the isthmus compared to ampullary region of the Fallopian tube (Table 1) though the difference between the various cyclic stages was not statistically significant. The values for the concentrations of E_2 and P are therefore expressed on the basis of tissue protein. There was, however, in both regions an overall good correlation between steroid concentrations expressed on weight basis and on the basis of tissue protein content ($r = 0.76$ to 0.96 for the two regions during the various phases). The steroid concentrations (amount per protein content) in the two regions are shown in Fig. 2. There was no significant difference in the concentrations between the ampulla and isthmus (paired t -test) at any of the menstrual phases.

The mean values for the tissue concentration of E_2 was highest both in ampulla and in isthmus around the time of ovulation. On the other hand, P showed a slight insignificant rise from the follicular to the ovulation period, whereas in the luteal phase a pronounced and significant increase was found (Fig. 2). There was a high degree of linear correlation between the ampulla and isthmus with regard to the concentration of E_2 and P ($r = 0.89$ and 0.97 , respectively) during the ovulation phase in contrast to the follicular ($r = 0.43$ and 0.54) and luteal ($r = 0.25$ and 0.04) phases. In five cases the steroid concentration was determined separately in the two tubes from individual patients (Table 2). No significant side-to-side difference was found irrespective of cyclic stage.

The ratios between tissue and plasma steroids in the two regions at the various menstrual stages are presented in Table 3. It can be seen for both the isthmus and ampulla that, whereas the capacity to accumulate E_2 was most prominent during the follicular phase, it was highest in the ovulation phase for P. As also shown in Table 3, this agrees with a high degree of linear correlation between the plasma and tissue values for P during the ovulation phase.

It was found that there was a tendency for an inverse relationship between the tissue/plasma ratio for P and the plasma level of the hormone in the individual patients. Assuming the tissue/plasma ratio of P reflected the amount of available P receptors, this ratio was plotted against the plasma P concentration in order to test the possibility of receptor inactivation. Linear regression analysis showed a fairly good correlation between the two parameters for both isthmus and ampulla during the follicular phase. This analysis was not performed for the ovulation phase owing to the interference by the high E_2 level, and it was not considered feasible during the luteal period when the levels of P are too high for detection of any variability in P receptors (cf. Fig. 2).

Discussion

The tissue concentration of E_2 and P was measured in the ampullary and isthmic regions of the human oviduct during the follicular, ovulatory and luteal phases of the menstrual cycle as defined on the basis of plasma steroid levels and confirmed by histological examination of the endometrium in cases where the operation also included hysterectomy. There is no previous data available on the content of E_2 or P concentration in the human tube. The concentration of either steroid in the tube reported here was largely similar to that reported for the human non-pregnant myometrium by Runnenbaum et al.³ and Batra et al.

(to be published). However, the P value for tubal tissue during the proliferative phase obtained in this study was about ten times higher than the corresponding myometrial value reported by Runnenbaum *et al.*,³ but in the same order of magnitude as recently found by us (Batra *et al.*, to be published). The discrepancy may be due to an inadequate extraction of P from the myometrial tissue.⁷ The cyclic fluctuations of the hormones in the tubal tissue followed the same pattern as that reported for the myometrium presented by Runnenbaum *et al.*³ in the myometrium. Our results show that in general the correlation between the tissue and plasma levels is poor, and that there was always a considerably higher steroid level in the tissue than in plasma, although the tissue/plasma ratio for the steroid concentrations varied during various phases. This suggests that the Fallopian tube can be considered as target organ for female sex steroids. Indeed, the existence of estrogen and progesterone receptors has recently been demonstrated in the Fallopian tube.^{12, 13}

A comparison of the hormone concentrations in the isthmus and ampulla is best made on the basis of protein content since this was different per unit wet weight in the two regions. The protein concentration was considerably higher in the isthmus which is not surprising in view of the fact that this is a more muscular tissue compared to the ampulla. In fact, the protein content in the isthmus compares well with that recently found for the myometrium.¹⁴ However, there was no significant difference in the protein concentration of either the ampulla or the isthmus in the various cyclic stages, which justifies the comparison of the steroid levels between different phases in the same tissue. According to this there was no statistically significant difference in either the E₂ or P level of the ampulla and isthmus in any of the phases, although the mean values for the ampulla were always slightly higher than for the isthmus.

The changes in the hormonal concentration in both tissues from one phase to another are best compared in relation to plasma levels, since the concentration in the blood is also changing during the menstrual cycle. We have therefore calculated the tissue/plasma ratio for such a comparison. This ratio was highest for both isthmus and ampulla in the follicular phase for E₂ and in the ovulatory phase for P.

The tissue/plasma ratio of the steroid concentrations can be considered to reflect the steroid accumulating ability of the tissue, which should be influenced to a large degree by the concentration of the receptors for each hormone. This should particularly be the case when the plasma concentrations are relatively low. When plasma hormone concentration is high so would the concentration in the tissues, but the contribution from binding of

hormone to non-specific sites (not receptors) in the tissue might be considerable under these conditions. Accordingly, in our data the receptor ability would be best expressed during the follicular phase for E_2 and during ovulation for P. In the luteal phase, however, besides the changes in the plasma concentration that would influence the tissue concentration, there might be additional effects of P on E_2 and P receptors. P has been shown to significantly reduce receptors for both E_2 and P in the uterus.¹⁵⁻¹⁷

The general conclusion from the present data is that although there was a similarity in the patterns of the cyclic fluctuations for the mean steroid concentrations in the tube and plasma, the correlation between the individual plasma and tissue values, as established in the linear regression analysis, was poor.

Acknowledgements

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TABLE 1. Protein content per gram tissue in the human Fallopian tube at different phases of the menstrual cycle. Mean values $\pm$ S.E.M., number of determinations within parenthesis.

Phase	Protein mg/g wet weight	
	Isthmus	Ampulla
Follicular	188.4 $\pm$ 16.9 (13)	95.3 $\pm$ 5.3 (13)
Ovulatory	143.1 $\pm$ 12.8 (8)	91.7 $\pm$ 7.2 (9)
Luteal	167.5 $\pm$ 12.8 (11)	97.0 $\pm$ 8.0 (11)

TABLE 2. E₂ and P concentrations (pg/mg protein) in right and left Fallopian tube from 5 women.

Isthmus E ₂		Ampulla E ₂		Isthmus P		Ampulla P	
right	left	right	left	right	left	right	left
3.9	2.5	1.6	1.1	39	38	40	38
2.5	1.9	1.9	2.1	54	50	54	54
4.6	3.7	2.7	3.0	61	43	43	55
8.1	8.0	10.1	12.5	68	64	63	74
3.0	2.0	3.0	3.0	48	37	38	38

Paired t-test showed no statistical significant difference between the concentrations in the right and left tube.

TABLE 3. Ratios between the tissue and plasma concentrations of E₂ and P in the Fallopian tube (mean $\pm$ S.E.M.) and the correlation coefficient (r) in the linear regression analysis of the tissue versus plasma concentrations of the steroids. Number of tissues analyzed = n.

Phase	n	Isthmus		Ampulla		Isthmus		Ampulla	
		E ₂ ratio	r	E ₂ ratio	r	P ratio	r	P ratio	r
Follicular	12	12.6 $\pm$ 2.0	0.03	7.8 $\pm$ 1.1	0.57	14.4 $\pm$ 2.7	0.50	8.9 $\pm$ 1.7	0.71
Ovulatory	8	6.7 $\pm$ 1.2	0.40	6.2 $\pm$ 1.0	0.39	25.7 $\pm$ 4.6	0.91	17.9 $\pm$ 4.0	0.81
Luteal	11	8.2 $\pm$ 1.4	0.21	4.8 $\pm$ 0.7	0.31	3.4 $\pm$ 0.5	0.53	2.3 $\pm$ 0.4	0.16

Legends to figures

Fig. 1 Plasma concentration of estradiol-17 β (A) and progesterone (B) during the follicular (Fol), ovulatory (Ov) and luteal (Lut) phases. Mean values $\pm$ S.E.M., number of determinations within parenthesis.

Fig. 2 Tissue concentrations of estradiol-17 β (A) and progesterone (B) during the follicular (Fol), ovulatory (Ov) and luteal (Lut) phases. Mean values $\pm$ S.E.M., number of determinations within parenthesis.

Fig. 3 Correlation between plasma progesterone and tissue/plasma progesterone ratio in follicular phase for isthmus (●) and ampulla (▲) in the linear regression analysis. The equations for the lines and the correlation coefficient (r) are given; The number of women from which the material was obtained is given within parenthesis.

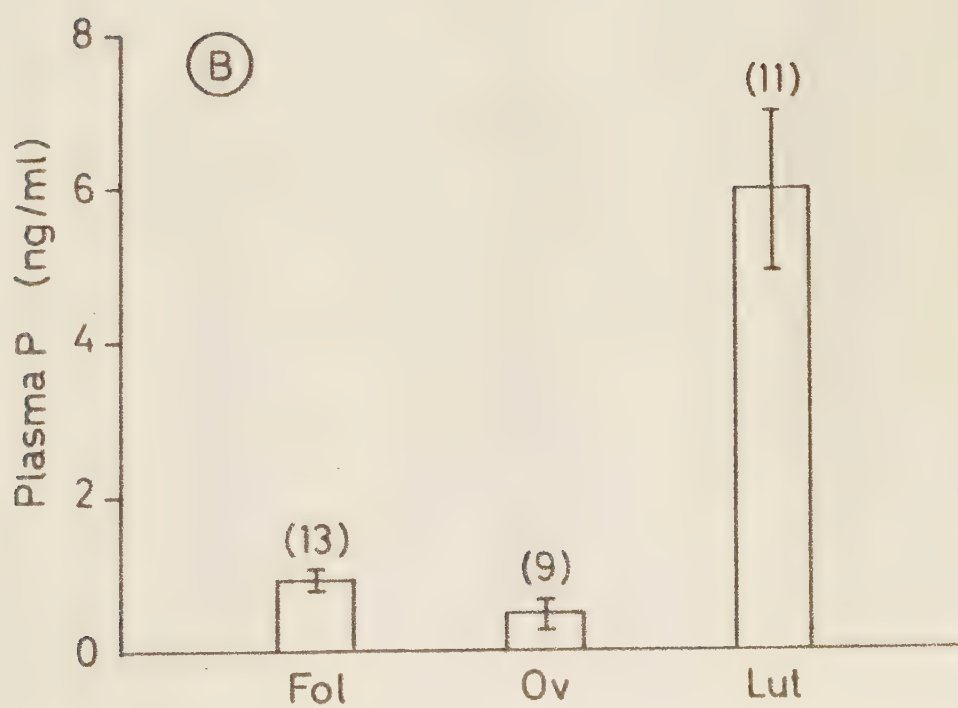
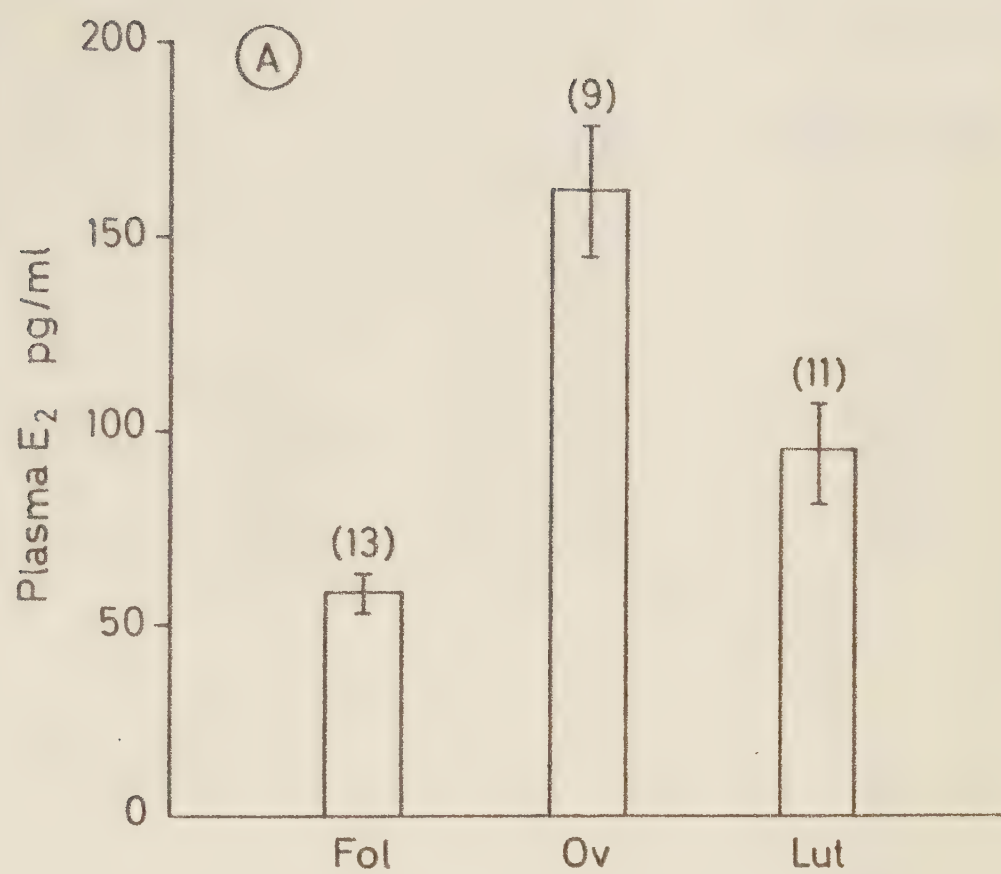


Fig. 1

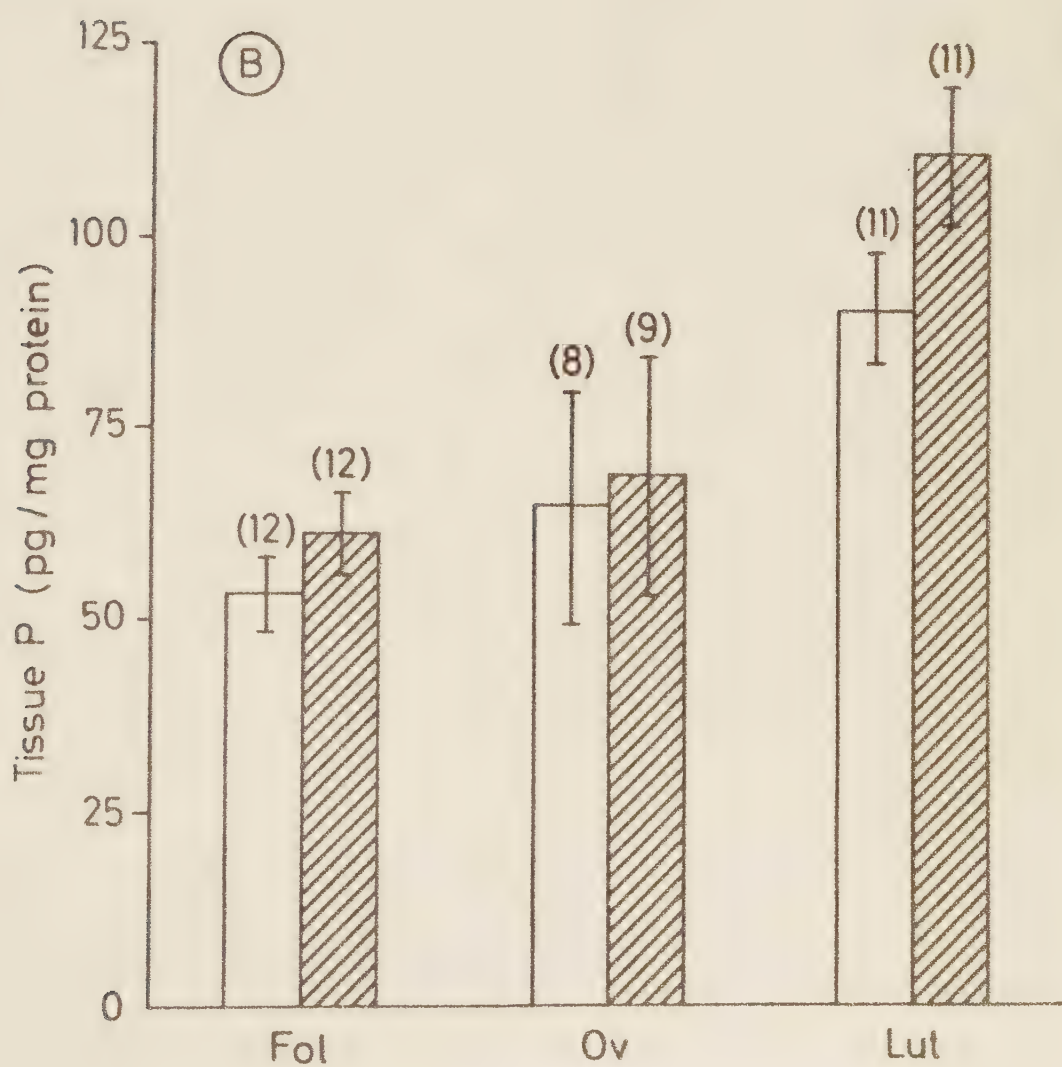
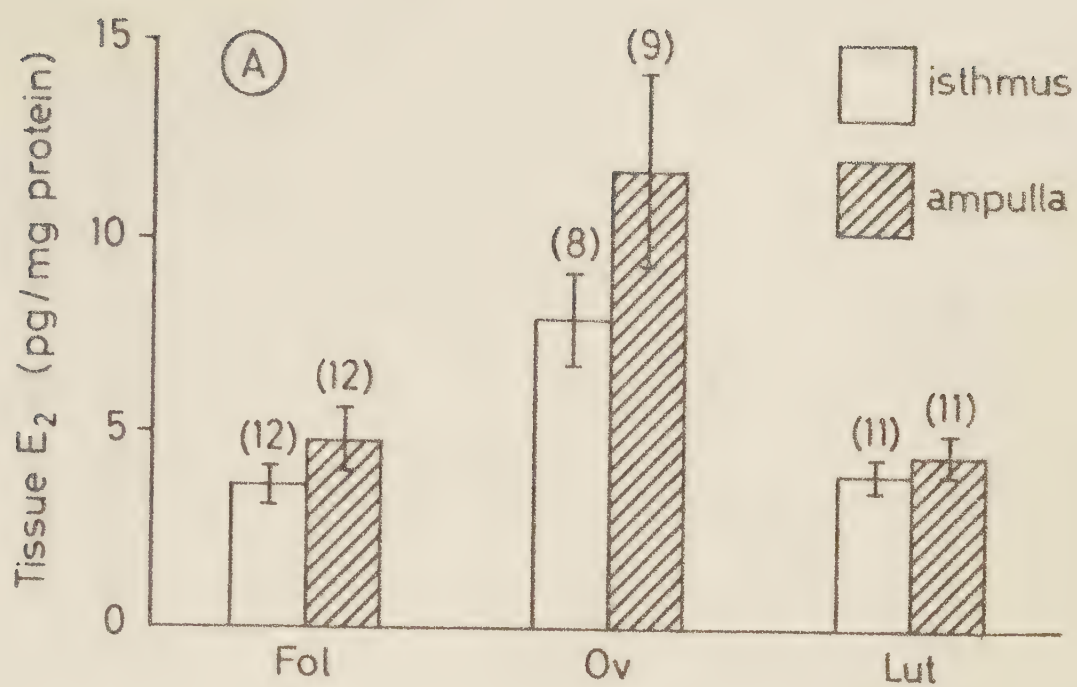
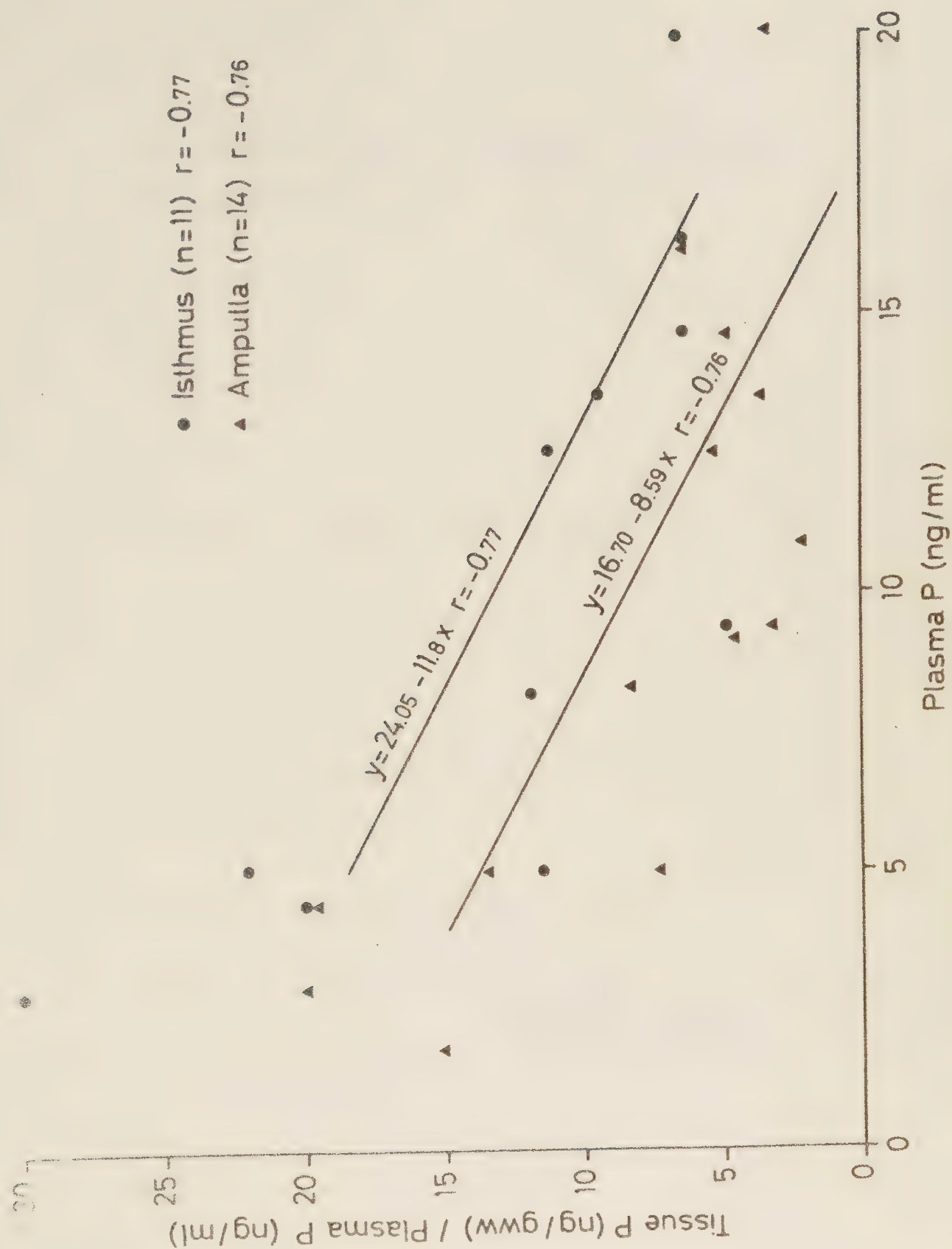


Fig. 2



SEX STEROIDS IN PLASMA AND REPRODUCTIVE TISSUES OF THE
FEMALE GUINEA-PIG

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ABSTRACT

Oestradiol-17 β and progesterone concentrations were determined in peripheral plasma, uterine, ovarian and placental tissues in non-pregnant (diestrous) and pregnant (mid- and full-term) guinea-pigs. Whereas there were relatively minor changes in plasma oestradiol concentration during pregnancy, plasma progesterone concentration increased by 70-fold at mid-term but decreased at term so that it was now 30-fold of that in the diestrous animals.

Uterine oestradiol concentrations nearly doubled at mid-pregnancy but decreased again at term to a value similar to that at diestrous. There was no significant change in uterine progesterone concentration during pregnancy. Ovarian oestradiol concentration in diestrous animals was about 10 times of that of the uterine concentration. At mid-term it increased 5-fold and further by 3-fold at term. Progesterone concentration in corpus luteum-containing ovaries was about 4 times greater than those not containing corpus luteum and was not significantly different than those in the pregnant animals. Placental oestradiol and progesterone concentrations were much lower than the ovarian concentrations of these steroids. Placental oestradiol concentration was even lower than the uterine oestradiol concentration whereas in the case of progesterone, the concentrations were 2-3 times higher in placenta than those in the uterus. The very low levels of progesterone in guinea-pig uterus and placenta might be explained by the secretion in plasma of progesterone binding globulin. However, why ovarian progesterone concentration remains very high is not clear.

Reproductive biology of the female guinea-pig has several features in common with man. Most important of these are firstly, that both guinea-pig and man have spontaneous ovulation with cyclic progesterone secreting corpus lutea, of both 14 days duration (Hilliard, 1973), secondly, removal of the ovaries after around mid-term of pregnancy does not affect the course of gestation (Sisk, 1976), and thirdly, parturition is not preceded by a fall in peripheral plasma progesterone (Illingworth, Challis, Ackland, Burton & Heap, 1974). The last two mentioned characteristics are in contrast to those in the rabbit and the rat (Hilliard, 1973), the other two mammalian species most commonly used as experimental animals in studies dealing with reproductive biology. Guinea-pig is therefore generally regarded as a most suitable animal model for elucidating steroid hormone dynamics and their role in parturition in humans (Sisk, 1976). However, there is one critical difference, often overlooked, in the reproductive endocrinology of guinea-pig and human which is that the guinea-pig plasma unlike human contains during pregnancy a specific and high affinity progesterone-binding globulin. Since it is the concentration of the free hormone in plasma that would largely determine the concentration of the hormone in the target tissue, one would expect to find a great difference in the concentration of progesterone in the target tissue, such as the uterus, in the guinea-pig and the human.

In previous studies we have reported data on the concentration of oestradiol-17 β and progesterone in the human and rabbit reproductive tissues (Batra, Grundsell & Sjöberg, 1977; Batra & Bengtsson, 1978; Batra, Öwman, Sjöberg & Thorbert, 1979; Thorbert, Batra, Öwman Rosengren & Sjöberg, 1976). In view of the abovementioned similarities

and dissimilarities in guinea-pig reproductive endocrinology to that in the human, we have, in the present study, examined the relationship between plasma and uterine levels of oestradiol and progesterone in both the non-pregnant and pregnant guinea-pigs. Furthermore, since there is some evidence showing that placental progesterone concentration in the guinea-pig is strikingly low (Heap & Deanesly, 1966), we have also determined the concentration of the above steroids in placental and ovarian tissues.

MATERIALS AND METHODS

Animals

Guinea-pigs of a mixed strain were used. Non-pregnant animals in the diestrous stage were killed on days 8-11 of the estrous cycle, determined as previously described (Thorbert, Alm, Owman & Sjöberg, 1977). Pregnant guinea-pigs were placed in two groups depending on the length of pregnancy, which was judged by palpation. After killing, under light anaesthesia, the day of pregnancy was checked by measurement of mean weight and mean crown-rump length of the fetuses (Draper, 1920; Kaufmann, 1969). The two groups were at 26-34 and 56-64 days of pregnancy referred below as mid-term and full term pregnancy, respectively. The following tissues were dissected out for tissue steroid analysis: one uterine horn, a piece of the placenta, and one ovary on the same side as the uterine horn. While under anaesthesia blood was drawn from the left heart ventricle and used for the steroid assay described below.

Steroid assays

Plasma. The procedure for radioimmunoassay of oestradiol-17 β was that described by Lindberg, Martinsson & Johansson (1974). The

antiserum was raised in sheep against oestradiol-17- α -6-oxime conjugated with bovine serum albumin and used in a dilution of 1:100 000. After extraction of 1 ml of plasma with diethyl ether, antiserum and ^3H -oestradiol-17 β was added. The mixture was equilibrated at 4°C overnight. The unbound steroid was removed by addition of dextran-coated charcoal. Extensive data on the specificity of the antiserum have been published previously by Lindberg et al. (1974).

This method used in previous studies on rabbit (Thorbert et al., 1976) and human (Batra and Bengtsson, 1978) plasma for the determination of progesterone (Youssefnejadian, Florensa, Collins & Somerville, 1972) was, although adequate for plasma from non-pregnant guinea-pigs, not satisfactory for progesterone determinations in pregnant guinea-pigs. Progesterone in these samples was therefore measured with the method for tissue determinations (see below) with the exception that only 3.5 ml of eluate from Sephadex LH-20 columns was collected. The antiserum (FO 22-5-73) which was a gift from Dr Kjell Martinsson and found to be highly specific for progesterone was used in a dilution of 1:1500 (Batra, Bengtsson, Grundsell & Sjöberg, 1976).

Tissues. The assay procedures for the determination of tissue progesterone and oestradiol-17 β have been reported previously (Batra, 1976; Batra & Bengtsson, 1976). Briefly, the frozen tissues were thawed and then digested in 0.5 ml of a mixture containing 5 % sodium dodecyl sulphate and 0.5 N NaOH. The digested material was extracted three times with 3 vol ethyl acetate. The combined extracts were evaporated to dryness under air at 40°C. Removal of sodium dodecyl sulphate from the extracted samples before radioimmunoassay was accomplished by Sephadex LH-20 gel chromatography. After evaporation of the collected (6 ml) eluate, the residue was dissolved in 1 ml ethyl acetate and suitable amounts, depending on the pre-

dicted concentrations of oestradiol and progesterone, were taken for their respective radioimmunoassay. Extensive data on the reliability of these methods have been reported previously (Batra, 1976; Batra & Bengtsson, 1976). Almost complete recoveries of oestradiol and progesterone added to digested placental and ovarian tissues were obtained.

Free and bound progesterone Free and protein-bound progesterone was measured by the membrane filter (Millipore) technique described previously (Batra, 1974; Batra et al., 1976).

Statistics

In the text and in tables values from multiple observations are given as means $\pm$ standard error of the mean; number of observations when given is shown within the parenthesis.

Student's t-test was used in the evaluation of observed differences in mean values.

RESULTS

When plasma progesterone from pregnant guinea-pigs was extracted with petroleum ether as done successfully previously for human and rabbit plasma, it was found that the solvent extracted 37-91 % ($n = 8$) of tritiated progesterone added to guinea-pig plasma. However, when plasma was first treated with SDS and NaOH as used for tissue steroid analysis, the recovery of added tritiated progesterone was 97.9 ± 1.28 % and the coefficient of variation ($n = 16$) was 10.4 %.

Incomplete and variable extraction of progesterone from pregnant guinea-pig plasma was probably due to the presence of high affinity

progesterone binding globulin (PBG). A measurement of the free and bound fraction of plasma progesterone (Table 1) showed that virtually 100 % was in the bound form in the pregnant guinea-pig whereas in the non-pregnant the mean value was 77.6 %.

Plasma

Oestradiol concentrations in plasma decreased slightly but insignificantly at mid-term of pregnancy (Fig. 1). However, at term it increased again and was significantly higher than that at mid-term ($p < 0.01$) or that in the diestrous animals ($p < 0.05$). Plasma progesterone concentration in the diestrous animals was of the order of 4 ng/ml and it increased by 70-fold at mid-term but decreased at term of pregnancy so that it was now 30-fold of that in the diestrous animal. The decrease from mid-term to term was significant ($p < 0.05$).

Uterine tissue

Oestradiol concentration nearly doubled ($p < 0.05$) at mid-term but decreased again at term to a value similar to that in the diestrous animals (Fig. 2). There was relatively little change in uterine progesterone concentration during pregnancy, the slight increase seen at mid-term was not significant.

The uterine/plasma ratio for oestradiol was much higher than unity at all times whereas for progesterone this ratio was about 6, 0.2 and 0.3 for diestrous, mid and full pregnant animals respectively.

Ovarian tissue

Ovarian oestradiol concentration in diestrous animals was about

10 times of that in the uterine tissue (Fig. 3). At mid-term it increased by 5-fold and further increased by 3-fold at term. There was no difference in the ovarian oestradiol concentration in the diestrous animals whether or not corpus lutea were present. However, progesterone concentration as expected was much higher (4 times) in corpus luteum containing ovaries than those not containing corpus luteum. But, progesterone concentration even in ovaries not containing corpus luteum was very high (25 times) compared to the uterus. Progesterone concentration in corpus luteum bearing ovaries of the pregnant animals was slightly higher than those of the non-pregnant animals but the difference was not significant.

Placental tissue

The placental oestradiol and progesterone concentrations were much lower than the ovarian concentration of these steroids (Fig. 3, Fig. 4). In the case of oestradiol the placental concentration was even lower than the uterine concentration, whereas in the case of progesterone, the concentrations were 2-3 times higher in placenta than in the uterus. When pregnancy advanced from mid-term to term, there was about 30 % reduction ($p < 0.005$) in both estradiol and progesterone concentrations.

DISCUSSION

Guinea-pig reproductive endocrinology is generally thought to correspond more closely to that of the human than of other mammalian species (Sisk, 1976; Thorburn, Challis & Robinson, 1977). The aim of the present study was to provide information of steroid dynamics in the guinea-pig reproductive tissues and to compare these with other mammalian species particularly the man. For comparison of the present

data with those obtained in other species, most of which are of recent origin, they are depicted in Table 1.

Plasma

Since plasma oestradiol concentrations changed relatively little during pregnancy they were extremely low when compared to those existing in human pregnancy. In this regard the guinea-pig is very different from human but rather similar to rabbit.

Plasma progesterone concentration increased by several orders of magnitude during pregnancy in the guinea-pig and showed its maximum around mid-term. It decreased by about 50 % at term. This pattern and the values for progesterone in the present study are in agreement with the data published previously (Heap & Deansley, 1966; Challis, Heap & Illingworth, 1971; Croix & Franchimont, 1975; Lea, Bessesen & Støa, 1976). Plasma progesterone levels in the guinea-pig at term are similar to those in man. However, in the former this progesterone is present virtually all in the bound form whereas in the latter about 10 % is free (Batra et al., 1976).

Uterine tissue

Oestradiol concentration in the guinea-pig uterus although being highest at mid-term, the changes during pregnancy were relatively small. This is consistent with the changes observed in plasma oestradiol.

What is most remarkable in the data of the present study is that the concentration of uterine progesterone did not change during pregnancy in spite of 30 to 60-fold increases in the concentration

of plasma progesterone. This situation is very much unlike that encountered in the human where uterine progesterone concentration keeps up well with the increases in plasma progesterone, at least up to mid-term (Batra and Bengtsson, 1978). As a result, the uterine progesterone concentrations in the human pregnancy at term are about 20-fold higher than in the guinea-pig in spite of similar plasma progesterone in the two species (see Table 1).

There are two factors at least that could well account for the very low levels of uterine progesterone in the pregnant guinea-pig. Firstly, plasma progesterone is almost all bound to a progesterone-binding protein occurring more or less specifically in the pregnant guinea-pig plasma (Milgrom, Allouch, Atger & Baulieu, 1972; MacLaughlin & Westphal, 1974; Lea et al., 1976). This protein (PBG) has been found to have a very high affinity for progesterone (Milgrom et al., 1972; MacLaughlin & Westphal, 1974; Lea et al., 1976) and our measurements showed that the progesterone in pregnant guinea-pig plasma was all in the bound form. These observations indicate that the bound progesterone cannot enter the uterus and clearly argue against the hypothesis that PBG might be involved in the transport of progesterone to the uterus (Lea et al., 1976).

The second reason for the very low progesterone concentration in the pregnant guinea-pig uterus might be the very potent inactivating effect of progesterone in this species on the uterine progesterone receptors (Milgrom, Luu Thi, Atger & Baulieu, 1973; Freifeld, Feil & Bardin, 1974; Baulieu, 1975). Recent data from this laboratory have shown that although there is a reduction in progesterone receptors after progesterone treatment in the rabbit

uterus, this reduction was not as dramatic as that reported for the guinea-pig (Batra, 1979). Similar evidence seems to exist for the human uterine progesterone receptors (Ellingworth, Wood, Flickinger & Mikhail, 1975; Damilano, Robel & Baulieu, 1978). One reason for the high inactivating effect of progesterone on its own receptors in the guinea-pig uterus might be attributed to very low levels of uterine (and plasma) oestradiol. Oestradiol would tend to, by its ability to promote the synthesis of progesterone receptors, compensate to some extent the inactivation by progesterone. Since uterine oestradiol concentrations in guinea-pig are exceedingly low, inactivation by progesterone of its receptors would be expected to be very effective and probably not easily reversible. There is some evidence that recovery of progesterone receptors after their inactivation by progesterone was much slower in the guinea-pig uterus than that in the chick oviduct (Mester & Baulieu, 1977).

Ovarian tissue

The very high concentrations of both steroids in the ovarian tissue is not surprising in view of the fact that the hormones are synthesized in the tissue. But this also indicates that the ovary unlike placenta has a high capacity to retain these hormones (see below). The fact that there was no difference in the concentration of oestradiol in contrast to progesterone in ovaries with and without corpus luteum indicates that corpus luteum in the guinea-pig unlike that in the human does not synthesize oestradiol. The large increase in the ovarian oestradiol concentration at the end of pregnancy is well reflected in the plasma concentrations.

With regard to progesterone concentration in the ovarian tissue it is most remarkable that the progesterone concentration in the ovary without corpus luteum was also very high, being 25 times greater than uterine progesterone concentration. Assuming that the corpus luteum is the sole source of progesterone in the ovaries, one will have to conclude that the very high concentrations of progesterone in the ovary not containing corpus luteum reflects an accumulation from the peripheral circulation. If this was the case, the present data would suggest the presence of a very high affinity progesterone binder in the ovarian tissue. The affinity for progesterone of this hypothetical binder would be much greater than that of the uterine tissue since the uterine tissue was unable to concentrate progesterone owing to the presence of the high affinity progesterone binding globulin in the plasma (see above). Alternatively the ovarian progesterone receptor may not, unlike the uterine receptors, be inactivated by progesterone. Interestingly oestradiol receptors in the rabbit corpus luteum similar to the uterine oestradiol receptors have been reported (Lea, Keyes & Jacobson, 1971).

It is also interesting to note that whereas ovarian progesterone concentration during pregnancy did not increase significantly from that found in corpus luteum-bearing ovaries of the diestrous animals, the plasma progesterone increased by many folds. This might be due to a substantial contribution by the placenta (Illingworth & Challis, 1973) and a considerable decrease in the metabolic clearance rate (Challis et al., 1971; Lea et al., 1976) resulting from progesterone binding to PBG.

Placental tissue

The concentration of either steroid in the placental tissue was surprisingly low compared to the ovarian tissue or human placenta tissue (Runnebaum, Runnebaum, Stölzer & Zander, 1975; Batra, Bengtsson & Sjöberg, 1979). Oestradiol concentration in placenta was in fact about 50 % of that in the uterine tissue. These data might suggest that the guinea-pig placenta does not synthesize oestradiol to any great extent, which is in agreement with the recent finding of Kalloo and Bhavnani (1978) who showed that the major site of oestrogen formation in both pregnant and non-pregnant guinea-pig was the ovary. The possibility that the very low concentrations of oestradiol in the guinea-pig placenta simply reflect contamination from the blood cannot be completely ruled out.

Surprisingly we also found very low concentration of progesterone in the placental tissue (Heap & Deanesly, 1966). These concentrations were about twice of that found in the uterine tissue but were very much lower than those in the ovarian tissue or human placental tissue (Runnebaum et al., 1975; Batra et al., 1979). Guinea-pig placenta as that of the human is known to synthesize substantial amounts of progesterone since ovariectomy after 20-25 day post coitum does not result in any significant decrease in the level of peripheral plasma progesterone, nor does it affect the course of pregnancy (Heap and Deanesly, 1966). Progesterone concentration in term placentae was lower than in mid-term placentae which is also reflected in the plasma progesterone levels. This observation lends further support to the evidence that plasma progesterone is mainly derived from the placenta after mid-pregnancy. We are, however, unable to provide an explanation for the very low

levels of progesterone in the placental tissue. It may be the result of very high binding of progesterone in the plasma but why this does not apply to the concentration in the ovarian tissue (see above) which contained a very high progesterone concentration, is not clear. There is also a suggestion that PBG originates in placenta (Milgrom et al., 1973; Lea et al., 1976) which adds further complexity to this problem.

The general conclusion that can be drawn from the present study is that the concentrations of oestradiol and progesterone in the uterine tissue, one of the most important targets for these steroids, and in the placental tissue in the guinea-pig, in comparison with the human, are very low. Although oestradiol levels in the peripheral plasma are very low compared to the human, the progesterone levels are similar. The very low levels of progesterone in the uterine tissue, and also in placenta could be explained on the basis of the PBG present specifically in the guinea-pig plasma during pregnancy (Milgrom et al., 1973; Lea et al., 1976). Thus the presence of PBG not only protects this steroid from metabolizing (Challis et al., 1971) but keeps it from accumulating in the target tissues. The biological significance of the latter is not yet clear.

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Table 1. Concentrations of free and protein-bound progesterone
in plasma of non-pregnant and pregnant guinea-pigs.

Reproductive state	Total P ng/ml	Free P ng/ml	Per cent bound	n
Non-pregnant	3.61 $\pm$ 0.69	0.76 $\pm$ 0.10	77.6 $\pm$ 2.57	4
Pregnancy (mid- and full term)	160.0 $\pm$ 39.40	0.51 $\pm$ 0.28	99.6 $\pm$ 0.23	9
Difference: Statistical significance	p < 0.005	N.S.	p < 0.001	

Table 2. Plasma and uterine tissue oestradiol and progesterone concentration in guinea-pig, man and rabbit in non-pregnant and pregnant (mid- and full term) state.*

Species	Sample	Estradiol pg/ml, g		Progesterone ng/ml, g			
		Non-pregnant	Mid-term	Full term	Non-pregnant	Mid-term	Full term
Guinea-pig	Plasma	29.8 [±] 6.0	20.5 [±] 4.5	59.3 [±] 8.9	3.6 [±] 0.7	203 [±] 37.1	102.0 [±] 13.1
	Uterine tissue	399.0 [±] 55.1	705.0 [±] 97.5	387.0 [±] 33.0	19.4 [±] 1.0	28.6 [±] 4.4	20.6 [±] 0.9
Man	Plasma	190.0 [±] 56.6	(1) 2900.0 [±] 1000.0	(2) 22000.0 [±] 1600.0	(2) 10.5 [±] 2.3	(1) 27.2 [±] 8.4	(2) 175.0 [±] 11.3
	Uterine tissue	607.0 [±] 104.0	(1) ^{**} 1600.0 [±] 600.0	(2) 3200.0 [±] 310.0	(2) 21.7 [±] 4.8	(1) 57.7 [±] 16.9	(2) 92.0 [±] (2)
Rabbit	Plasma	31.8 [±] 4.0	(3) 60.0 [±] 6.0	(5) 50.0 [±] 10.0	(5) 0.3 [±] 0.1	(4) 18.0 [±] 2.0	(5) 7.0 [±] 1.0
	Uterine tissue	950.0 [±] 175.0	(3) 550.0 [±] 90.0	(5) 950.0 [±] 200.0	(6) 6.1 [±] 0.3	(4) 20.0 [±] 4.0	(6) 4.0 [±] 1.0

* Data in guinea-pig are those reported in the present study whereas in man and rabbit are those reported in studies indicated by a reference number in parenthesis.

** Endometrial tissue but values are comparable to those recently obtained for the myometrium (Batra et al. unpublished)

1. Batra et al. (1977),
2. Batra and Bengtsson (1978),
3. Batra et al. (1979),
4. Thorbert et al. (1976),
5. Challis et al. (1973),
6. Challis et al. (1974)

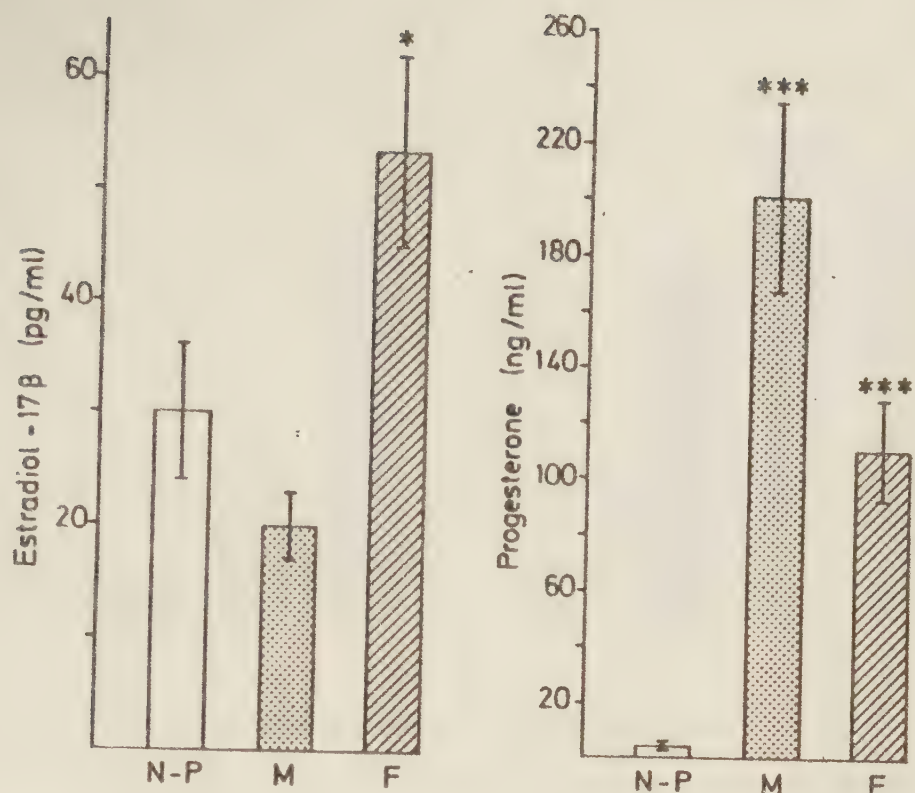


Fig. 1 Oestradiol and progesterone concentration in peripheral plasma of nonpregnant (N-P), mid-term (M), and full term (F) pregnant guinea-pigs. Significance of difference from N-P is indicated: * $p < 0.05$, *** $p < 0.005$.

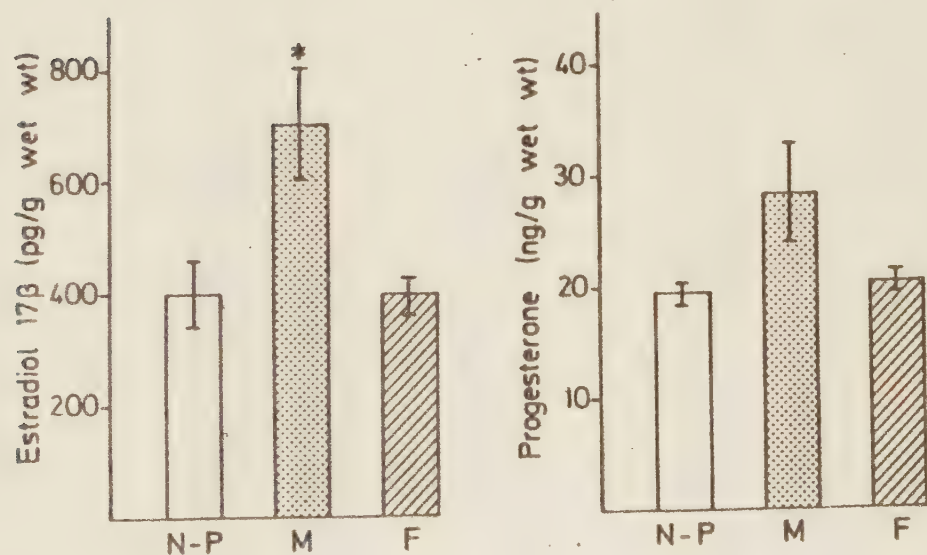


Fig. 2 Oestradiol and progesterone concentration in uterine tissue from nonpregnant (N-P), midterm (M), and full term (F) pregnant guinea-pigs. Significance of difference from N-P is indicated by* $p < 0.05$.

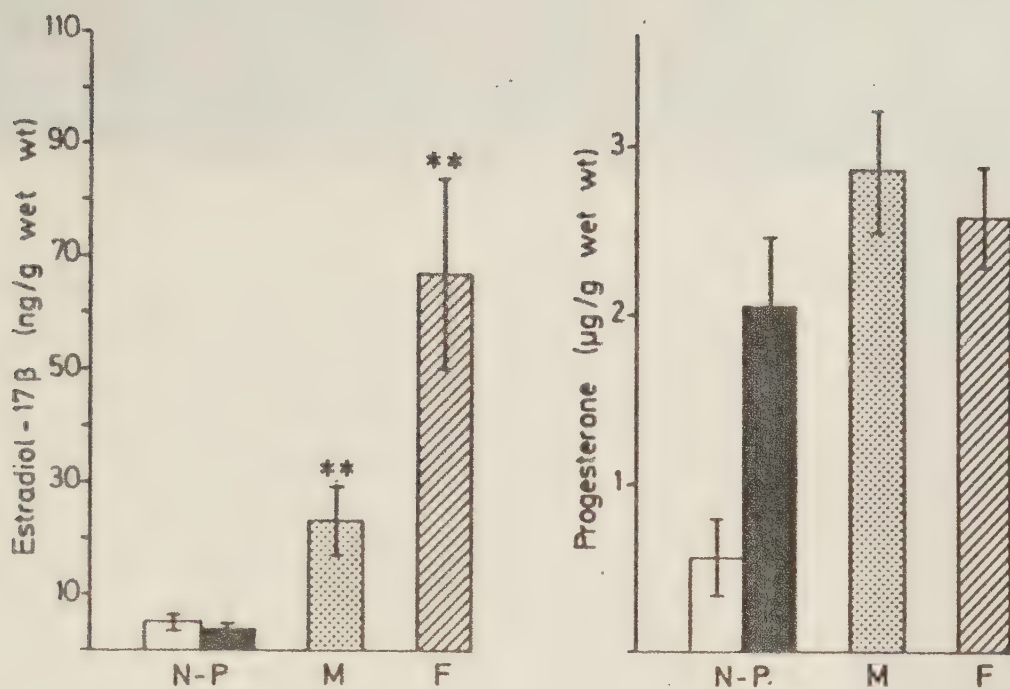


Fig. 3 Oestradiol and progesterone concentration in ovaries from non-pregnant (N-P), mid-term (M) and full term (F) pregnant guinea-pigs. Filled columns represent corpus luteum containing ovaries. Significance of difference from N-P is indicated by ** .P < 0.01.

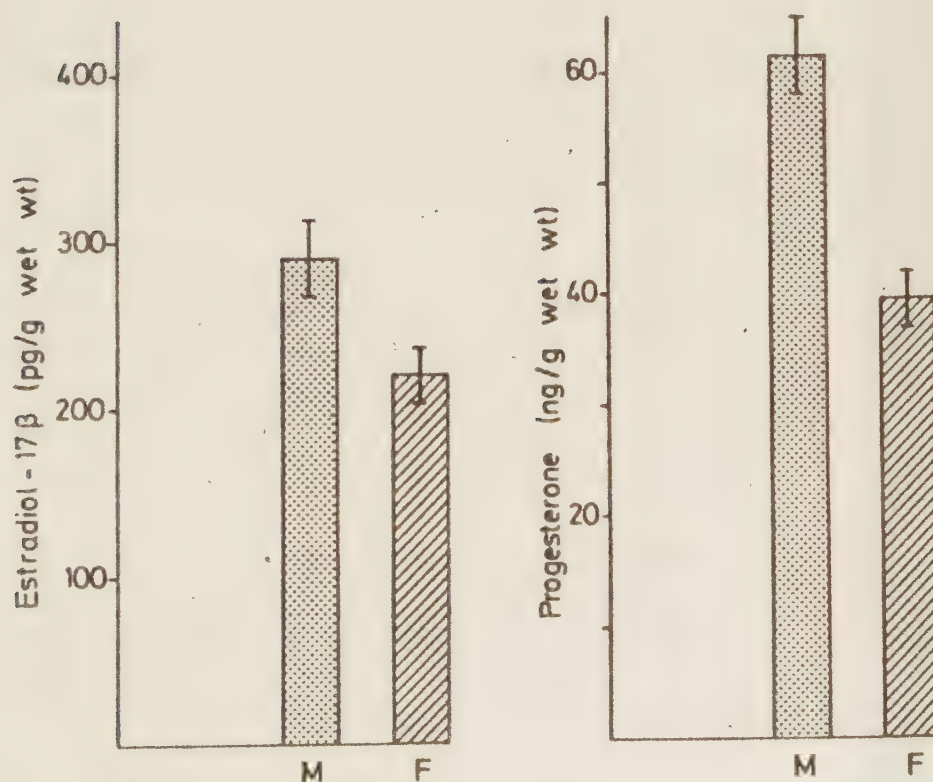


Fig. 4 Oestradiol and progesterone concentration in guinea-pig placenta at mid-term (M) and full term (F) of pregnancy.

ent, some insight into these mechanisms could be obtained. The detailed data of these investigations are reported in paper XIV.

A considerable reduction in the cytosolic P receptors in the rabbit myometrium after progesterone treatment could clearly be demonstrated. However, the reduction is not as marked as that shown in the guinea-pig. Neither stripping the cytoplasm of endogenous P nor loading it with P in vitro led to any increase or decrease in P receptors. These data indicate that occupation of the receptors will not influence receptor determination, unless it is postulated that when P binds endogenously to a receptor, it does not exchange as readily as when bound under in vitro conditions.

In order to recover cytoplasmic receptors from nuclei, nuclear pellets were washed with buffered sucrose solutions and the washings collected. Although the P concentration in the cytoplasm so extracted from the nuclear pellets of P-exposed uteri was significantly higher, the receptor concentration was lower than in similar extracts from untreated animals. Furthermore, the concentration of nuclear receptor (extracted with 0.4 M KCl) did not differ significantly between the P treated animals and the controls. Thus, it appears unlikely that the reduction of cytoplasmic P receptors was solely due to the translocation in the nucleus (35,38). P treatment also resulted in a reduction of uterine E2 receptors which confirms data of previous studies (12,25). The data on P distribution in the subcellular fractions showed that after P treatment the P concentration increased significantly in the cytosol as well as in the intact tissue. The increase in P concentration in other fractions was not significant. Treatment caused no change in the subcellular distribution of protein, but there was a marked change in the distribution of P. An insignificant decrease in tissue E2 and a significant decrease in the cytosolic E2 concentration was observed after P treatment, which supports our previous data showing that uterine E2 concentration is regulated more by the existing tissue P concentration rather than by the plasma E2 concentration (XI).

The P concentration per mg protein was highest in the mitochondrial + microsomal fraction although the protein content of this fraction per unit weight of the tissue was lowest. Since the mitochondrial + microsomal fraction of the present study will also contain the lysosomal fraction, the high concentration of both E2 and P in this fraction might be taken to support recent suggestions on the presence of steroid hormone receptors in uterine lysosomes (55,61). However, further data will be needed to verify this point.

Although the data of the present study could not explain the mechanism underlying the reduced disappearance of P receptors, evidence could be given against concepts based on the occupation of endogenous receptors or the translocation of receptors from the cytoplasm to the nucleus. The extreme stability of the progesterone-receptor complex formed in vivo, in contrast to that formed in vitro may be one reason why the

cytosol contains a high concentration of P after P treatment despite a reduced concentration of P receptors.

Guinea-pigs

Reproductive endocrinology of the female guinea-pig has several features in common with that of man, which are not found in the rabbit and the rat (see 56 for references). For this reason guinea-pig is generally regarded as the most suitable animal model for elucidating steroid hormones dynamics and their role in parturition in man (56). Further studies were therefore performed on guinea-pig reproductive tissues (XV).

The data showed that the E2 and P concentrations in reproductive tissues, particularly the placenta and the uterus, were strikingly different from those in the corresponding human tissues. For easy comparison, the concentrations in the plasma and the uterus of the rabbit, guinea-pig, and man are given in Table I.

Since the plasma E2 concentration changes relatively little during pregnancy in the guinea-pig it was extremely low when compared to that occurring in human pregnancy. Accordingly, the concentration in the uterine tissue was also much lower than that observed in man. Plasma P levels in guinea-pig pregnancy were similar to those in human pregnancy except that in man the peak level was reached at term, whereas in the guinea-pig the highest levels were found around mid-term.

It is noteworthy that the uterine P concentration did not change significantly during pregnancy in the guinea-pig in spite of 30-70-fold increases in the plasma P concentration. This situation is quite unlike that encountered in man, where uterine P keeps up well with increases in plasma P, at least until mid-pregnancy (VIII and IX).

There are at least two factors that could account for the very low levels of uterine P in the pregnant guinea-pig. Firstly, almost all of the plasma P is bound to a P binding globulin (PBG) occurring specifically in pregnant guinea-pig plasma (32,40). The present data show that almost all P in plasma was in the bound form. In human pregnancy about 10 % of plasma P is free (IV) and in the non-pregnant guinea-pig more than 20 % is free (XV). The second reason for the low uterine P in the pregnant guinea-pig might be the potent inactivating effect of P on P receptors in this species (35,41). It was shown in a previous study (XIV) that, although P treatment led to a reduction in P receptors in the rabbit uterus, this reduction was not as dramatic as that reported for the guinea-pig uterus. The available data on the human uterine P receptors indicate that the P-induced reduction is much smaller than that observed in the guinea-pig uterus (6).

The placental E2 and P concentrations in the guinea-pig placenta were exceedingly low when compared to the human placenta or the guinea-pig ovarian tissue. The E2 concentration in the placenta was about 50 % of that in the uterine tissue. This might

Table I. Plasma and uterine tissue oestradiol and progesterone concentrations in guinea-pig, man and rabbit in the non-pregnant and pregnant (mid- and full term) state.*

Species	Sample	Oestradiol pg/ml, g			Progesterone ng/mL, g		
		Non-pregnant	Mid-term	full term	Non-pregnant	Mid-term	Full term
Guinea-pig	Plasma	29.8 [±] 6.0 (XV)	20.5 [±] 4.5 (XV)	59.3 [±] 8.9 (XV)	3.6 [±] 0.7 (XV)	203.0 [±] 37.1 (XV)	102.0 [±] 13.1 (XV)
	Uterine tissue	399.0 [±] 55.1 (XV)	705.0 [±] 97.5 (XV)	387.0 [±] 33.0 (XV)	19.4 [±] 1.0 (XV)	28.6 [±] 4.4 (XV)	20.6 [±] 0.9 (XV)
Man	Plasma	190.0 [±] 56.6 (3)	2900.0 [±] 1000.0 (VIII)	22000.0 [±] 1600.0 (VIII)	10.5 [±] 2.3 (3)	27.2 [±] 8.4 (VII)	175.0 [±] 11.3 (VIII)
	Uterine tissue	607.0 [±] 104.0 (3)**	1600.0 [±] 600.0 (VIII)	3200.0 [±] 310.0 (VIII)	21.7 [±] 4.8 (3)	54.7 [±] 16.9 (VIII)	92.2 [±] 6.8 (VIII)
Rabbit	Plasma	31.8 [±] 4.0 (XI)	60.0 [±] 6.0 (8)	50.0 [±] 10.0 (8)	0.3 [±] 0.1 (62)	18.0 [±] 2.0 (8)	7.0 [±] 1.0 (8)
	Uterine tissue	950.0 [±] 175.0 (XI)	550.0 [±] 90.0 (8)	950.0 [±] 200.0 (9)	6.1 [±] 0.3 (62)	20.0 [±] 4.0 (9)	4.0 [±] 1.0 (9)

* Roman numerals in parentheses refer to the original papers listed on page 4 whereas the Arabic numerals refer to those in the reference list on page 27.

** Endometrial tissue but values are comparable to those recently obtained for the myometrium (Batra et al. unpublished)

be explained by the existing evidence that the guinea-pig placenta, or the foetal placental unit, in a contrast to the human placenta does not synthesize E2 to any great extent (27,28). The very low P concentration in the guinea-pig placenta cannot easily be explained, particularly as there is evidence that the placenta of the guinea-pig synthesizes a substantial amount of P and that ovariectomy after 20-25 days post coitum does not result in any significant decrease in the level of peripheral plasma P; nor does it affect the course of pregnancy (22). The very low P concentration in the guinea-pig placenta might be explained by binding of P to PBG, but it is not clear why there is not a similar effect on the P concentration in the ovarian tissue, which is very high. It is also puzzling that the P concentration was relatively high in the ovarian tissue of dioestrous animals even in the absence of a corpus luteum.

GENERAL DISCUSSION

The data obtained in the first phase of the present study demonstrated that endogenous E2 and P concentrations could be reliably determined in small samples of reproductive tissue. In addition, a rapid, simple and reliable method could be developed for the determination of free P. This method was not satisfactory for the determination of free E2. Since E2 is not considered as important as P for the maintenance of pregnancy in man and since tissue E2 concentrations, in contrast to those of P, change relatively little during pregnancy in man and several other mammalian species (e.g. rabbit and guinea-pig), no rigorous attempts were made to develop a method for the determination of free and bound E2 concentrations in plasma.

The studies on human reproductive tissues clearly demonstrated a lack of relationship between tissue and plasma concentrations of E2 and P. This was particularly true for the myometrium and the decidua at full term pregnancy. Very low E2 concentrations in the myometrium relative to plasma during pregnancy can only be explained at the moment by the reduction in E2 receptors caused by P. However, this does not rigidly apply to P concentration in the tissue, although the P receptors have also been shown to be depressed by P. The tissue P concentrations were relatively high, and at mid-term they were considerably higher in both decidua and myometrium than in plasma. From the data on P concentrations in the myometrium and decidua, it can be concluded that there is a considerable binding of P in these tissues since otherwise, tissue P concentrations should be no greater than the concentration of free P in plasma, which was an order of magnitude smaller. Since P receptor might be expected to be at a very low level in tissues with high P concentrations, P is probably bound to sites (nonspecific) other than its receptors. If so, these nonspecific sites have a relatively high affinity for P. This kind of nonspecific binding of P might account for the considerable difficulty encountered in earlier studies in the measurement of specific progesterone receptors in the uterus as opposed to E2 receptors (2,19,37). There is also the possibility that the *in vitro* receptor measurements do not represent the true endogenous concentration, or more likely the characteristics, of the receptors formed *in vivo*. The present data on the measurement of E2 and P receptors in the rabbit uterus after P treatment seem to lend some support to this possibility.

It was possible to demonstrate that, although the cytosolic P concentration in the rabbit myometrium increased after P treatment, P receptor levels decreased markedly. The E2 concentration in the cytoplasm seemed on the other hand, to reflect changes in P receptor levels following P treatment. The tentative conclusion from these studies, until further data are available, must be that tissue E2 concentrations are predominantly governed by the concentration of E2 receptors. In rabbit and man, but not in guinea-pig the tissue P concentration reflects the plasma concentration better

than is the case with the tissue E2 concentration and bears an inverse relation to P receptor level. A similar trend is observed in the data on E2 and P concentrations in the human Fallopian tube (X).

The data on guinea-pig reproductive tissues show that E2 and P dynamics in the guinea-pig and man are different qualitatively as well as quantitatively. The uterine P concentration increased insignificantly during guinea-pig pregnancy, whereas in man the levels had risen by 10-fold by the end of the term. The total plasma P concentrations in guinea-pig and man at term pregnancy are fairly comparable, and in fact this is the only parameter in which the two species can be considered similar, as far as E2 and P concentrations in the various compartments are concerned. These data also show that a comparison between plasma concentrations of steroids in two different species can be quite misleading since in spite of similar concentrations in the plasma, the concentrations in the tissues may be remarkably different. It is reasonable to suppose that the biological activity of a hormone will be governed by its concentration in the tissue rather than in the peripheral plasma.

The very low concentration of P in the guinea-pig uterus could be explained by the presence of progesterone binding globulin (PBG) specifically present during pregnancy. The primary function of PBG is a matter for speculation. It is able to reduce the metabolic clearance rate to a great extent (7,32), and thereby conserves P, but at the same time, as shown by the present results, it completely inhibits P accumulation by the uterus. No such radical change takes place in human pregnancy and, as a result, uterine P concentrations increase substantially. However, the significance of these high uterine P concentrations, is also open to question, if it is accepted that the primary effects of P are mediated through its binding to the cytosolic receptor. These receptors will be saturated, as shown by our calculations, with tissue concentrations of about 1 ng/g. What the remaining (about 99 %) uterine P accomplishes during human pregnancy is not clear. It is possible that this remaining P has influence on other uterine functions such as the muscular contractility which are probably not mediated via classical receptor systems. However, there are no data in support of this and our preliminary data (Batra *et al*, unpublished) in fact show a lack of relationship between the *in vivo* myometrial activity and the myometrial P concentration.

One definite effect of the high concentration of uterine P, the functional significance of which must await further investigation, will be to reduce the E2 concentration and E2 receptor levels in the uterine tissue. A low uterine E2 concentration may be important for the proper physiological function of the uterus in pregnancy. In the guinea-pig and rabbit uterine E2 is low due to relatively little change in the plasma E2 concentration, while in man it is low because of depression of E2 receptors by the high P concentration in the tissue.

In conclusion the data of the present study have provided an overall survey of the

E and P concentrations in the various reproductive tissues of the female man, rabbit and guinea-pig. There are several factors that are important in the regulation of the tissue hormone concentration including the concentration of the free hormone in the plasma and the level of the hormone receptor in the tissue. Since, the hormones themselves control their receptor levels, a considerable complexity is added to a study of the mechanisms regulating steroid hormone concentrations in the target tissues. These mechanisms can be elucidated only when more detailed information on not only the concentrations of the hormones but also on their receptor in the tissue under various hormonal conditions are available.

SUMMARY

Methods are described for the determination of free and protein-bound progesterone in plasma and for the determination of oestradiol-17 β (E2) and P concentrations in the female reproductive tissues.

The levels of free (unbound) and protein-bound P were measured in serial blood samples obtained from the 23rd week of pregnancy until delivery. In addition, the levels of total plasma E2 in these samples were determined. Total and free P increased steadily until the end of pregnancy. Total E2 also increased with advancing pregnancy, but at no time prior to delivery was there an abrupt rise in E2 or a significant fall in P.

E2 and P concentrations were determined in plasma and in several reproductive tissues from pregnant and non-pregnant humans and guinea-pigs. In addition, plasma and tissue concentrations of these steroids were determined during pseudopregnancy in the rabbit, and after steroid administration in the non-pregnant rabbit. The relationship between the steroid hormone concentrations in the plasma and the tissues was analysed in each species, and a comparison was made particularly between man and guinea-pig. To elucidate the mechanism of hormonal control of the hormone receptor concentration, the subcellular distribution of E2 and P as well as the concentration of their receptors, were determined in the myometrium of the rabbit. The main findings of these investigations can be summarized as follows.

Plasma E2 levels in the guinea-pig, in contrast to those of human, changed little during pregnancy.

Plasma P levels increased very markedly (17-56-fold) during pregnancy in both man and guinea-pig.

There was a substantial increase in uterine E2 during human pregnancy, so that at full term the E2 concentration was about five times greater than in the non-pregnant uterus. However, the increase in uterine E2 was by no means parallel to that in plasma E2, which increased by more than 100-fold during the corresponding period. The changes in uterine E2 levels in guinea-pig pregnancy were relatively minor.

The uterine P concentration increased markedly during human pregnancy, whereas in the guinea-pig, it did not change significantly in spite of a 56-fold increase in the plasma P concentration.

Whereas the concentration of E2 in the human myometrium was significantly influenced by the distance from the placenta, the P concentration was not. This aspect was not examined in the guinea-pig.

The concentrations of both E2 and P in the guinea-pig placenta were much lower than those in the human placenta.

The uterine E2 concentration decreased significantly during pseudopregnancy in

the rabbit, in spite of relatively constant level in the plasma.

8. The administration of P to oestrogenized rabbits did not influence E2 levels in the plasma, but caused a significant reduction in uterine E2. Uterine E2 levels could again be elevated by simply withdrawing the influence of P.
9. Cytoplasmic P and E2 receptors in the rabbit myometrium decreased 24 hours after a single injection of P. However, the P concentration increased significantly both in the intact tissue and in the cytosol. Nuclear P receptor levels in the P treated and controls were similar.
10. After P treatment there was no change in the subcellular distribution of protein, but a marked change in the distribution of P.
11. An insignificant decrease in tissue E2 and a significant decrease in the cytosolic E2 concentration was observed after P administration.

The significance of the above findings is discussed with respect to the control of hormonal concentrations in the tissues and their possible role in parturition.

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